agronomy

MDPI

Article

Impact of Mycorrhizal Fungi from Different Rhizospheric Soils on Fungal Colonization, Growth, and Chlorophyll Contents of *Cenchrus ciliaris*

Sumaira Thind [1,2], Muhammad Shafiq Chaudhary [3], Allah Ditta [4,5,*], Iqbal Hussain [2], Abida Parveen [2], Naseer Ullah [6], Qaisar Mahmood [7,8], Ibrahim Al-ashkar [9] and Ayman El-Sabagh [10]

1 Cholistan Institute of Desert Studies, Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan
2 Department of Botany, Government College University, Faisalabad 38040, Pakistan
3 Department of Microbiology, University of Central Punjab Lahore, Lahore 54590, Pakistan
4 School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia
5 Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Upper Dir 18000, Pakistan
6 Environmental Chemistry Laboratory, Department of Environmental Science and Engineering, School of Space and Environment, Beihang University, Beijing 100083, China
7 Department of Environmental Sciences, COMSATS University Islamabad, Campus, Abbottabad 22060, Pakistan
8 Department of Biology, College of Science, University of Bahrain, Sakhir 32038, Bahrain
9 Department of Plant Production, College of Food and Agriculture, King Saud University, Riyadh 11451, Saudi Arabia
10 Department of Agronomy, Faculty of Agriculture, University of Kafrelsheikh, Kafr el-Sheikh 33516, Egypt
* Correspondence: allah.ditta@sbbu.edu.pk or allah.ditta@uwa.edu.au

Abstract: Mycorrhizae are symbiotic associations between fungi and plants and are primarily responsible for nutrient transfer and survival of both partners. The present study was conducted to explore the diversity of mycorrhizal fungi in the rhizospheric soil of perennial grass species (*Saccharum spontaneum*, *Saccharum bengalense*, *Setaria verticillata*, *Cymbopogon jwarancusa*, and *Typha angustata*) around the district Layyah. In the subsequent experiment, the rhizospheric soils were used as inoculants, and their impact on mycorrhizal colonization in the plant and soil, and growth and physiological attributes, of *Cenchrus ciliaris* were investigated. The maximum hyphal, vesicles, arbuscules, dark septate endophytic and ectomycorrhizal colonization, and spore percentage were observed in the case of R-S_5, i.e., rhizospheric soil, collected from *Saccharum bengalense*. However, the maximum (0.9310) Simpson's index of diversity was observed in the case of R-S_4, i.e., rhizospheric soil collected from *Setaria verticillata*. Different mycorrhizal fungal morphotypes scattered over three genera, i.e., *Acaulospora*, *Glomus*, and *Scutellospora*, were recorded both from rhizosphere and trap cultures. The application of spores from rhizospheric soil collected from *S. bengalense* (R-S_5) caused the maximum increase in plant height (19.5%), number of leaves plant^{-1} (17.6%), leaf area (108.0%), and chlorophyll contents (29.4%) of *Cenchrus ciliaris*, compared to other treatments. In conclusion, the inoculation of mycorrhizal fungi significantly improves the mycorrhizal characteristics of *Cenchrus ciliaris* and its rhizospheric soil and ultimately enhances the growth and physiological parameters of *Cenchrus ciliaris*.

Keywords: buffel grass; diversity; growth and physiological parameters; mycorrhizal colonization; Simpson's index; spores

check for updates

Citation: Thind, S.; Chaudhary, M.S.; Ditta, A.; Hussain, I.; Parveen, A.; Ullah, N.; Mahmood, Q.; Al-Ashkar, I.; El-Sabagh, A. Impact of Mycorrhizal Fungi from Different Rhizospheric Soils on Fungal Colonization, Growth, and Chlorophyll Contents of *Cenchrus ciliaris*. *Agronomy* **2022**, *12*, 2644. https://doi.org/10.3390/agronomy12112644

Academic Editor: Samantha C. Karunarathna

Received: 24 August 2022
Accepted: 18 October 2022
Published: 26 October 2022

1. Introduction

Mycorrhiza is a mutualistic relationship between soil-borne fungus and the roots of a wide range of plant species, including higher plants and perennial grass species [1]. Two types of mycorrhizal associations are known as ecto- and endo-mycorrhiza. The

ecto-mycorrhiza is characterized by an extracellular fungal growth in the root cortex [2,3]. Boreal forest trees have more than 5000 species, mainly of the Basidiomycetes variety, and are more common in the temperate zone [4]. The arbuscular mycorrhizal fungi (AMF) belong to the taxonomic order called Glomales, which currently comprises six genera and are the most common underground symbiotic fungi with agricultural applications [5,6]. AMF have been observed in all ecosystems [7]. Extra-radical AMF acquire carbon from the plant, transform it into storage lipids that take up mineral nutrients from the soil, and then transfer them to the plant roots; in the opposite direction, carbon is exported to build spores and mycelium from inter radical mycelium [8–10].

Arbuscular mycorrhizae are the associations where a Glomeromycete fungus produces arbuscules, hyphae, and vesicles within root cortex cells [11]. These associations are due to the presence of arbuscules. Fungi in the roots are usually spread by linear hyphae or coiled hyphae [12]. An important feature of the AM fungi is the production of a large number of soil-borne spores having hundreds or thousands of nuclei, presumed asexuality, and multinucleate mycelium without true septa [1]. Over 200 species of fungi are capable of forming a mycorrhizal association with the majority of plants.

Symbioses among dark septate endophytes (DSE) and AMF in terrestrial environments are ubiquitous, giving tolerance against harsh soil conditions that may limit plant growth [13]. Symbioses with AMF are especially crucial for the uptake of slow-moving soil nutrients [1]. In nature, DSE and AMF colonize plant roots at the same time, which is frequent in plant ecosystems [14]. Despite the presence of DSE and AMF in plant roots, there has been little research into these symbioses as a whole, and existing information regarding DSE and AMF is uneven [15]. Although microscopic or molecular analyses of roots generally showed that DSE could be more abundant than AMF, little is known about the role of DSE symbiosis on plant fitness [16].

Mycorrhizal fungi colonize approximately 85% of the plants, which suggests that mycorrhizal symbiosis is the rule rather than the exception in the plant world [17]. Root colonization by mycorrhiza improved nutrient uptake; increased tolerance against pests and diseases, drought, and heavy metals; and had a significant impact on the development and health of host plants [18–20]. Most ectomycorrhizal fungi break down phenolic compounds in soil that may obstruct nutrient absorption [21]. Vesicular arbuscular mycorrhizal (VAM) and ectomycorrhizal (ECM fungi) can protect roots from nematodes and parasitic fungi [22]. Hyphal networks help in the seedling establishment or contribute to the growth via the provision of nutrients, especially when roots are inactive [1]. Mycorrhizal fungal hyphae are also an important food source for soil invertebrates [23]. The inoculation of AMF enhanced the growth, production, and phosphorus uptake in *Setaria splendida* [1,24].

Buffel grass (*Cenchrus ciliaris* L.) is valued for its production of excellent feed and intermittent grazing during drought periods in the tropics. It is also highly nutritious and regarded as appropriate for pasture in hot and dry environments [25]. It grows in dry and sandy locations with annual rainfalls of 250–750 mm (but it may survive much higher rainfall) between sea level and 2000 m, on marginally fertile shallow soils [25]. Such qualities boost its value as pasturage and broaden its spectrum of production. Some strains' yield makes them suitable for foraging during the wet season. On a dry matter basis, buffel grass contains proteins (11.0%), fats (2.6%), total carbohydrates (73.2%), fibers (31.9%), and ash (13.2%) [26]. It is believed that feeding cattle green grass, and turning it into silage or hay, can boost their milk production, and give them a sleek, glossy appearance [26].

Mycorrhizae also influence soil microbial populations and exudates in the hyphosphere and mycorrhizosphere [27,28]. VAM fungal hyphae improve soil structure. Their importance in mechanical aggregation is due to the secretions of glomalin [29,30]. There is meager knowledge regarding the presence of mycorrhizal fungi in the desert ecosystem of Pakistan [31]. Nothing is known about the hidden potential for colonization status and AMF plant interaction, community formation, diversity, propagules, behavior, and spatial variations, in this region of Pakistan. Therefore, it is pertinent to explore the potential of different mycorrhiza in improving the growth and productivity of grasses, such as *Cenchrus*

ciliaris, as these serve as a food source for different animals. Based on this hypothesis, the purpose of the present study is to investigate the diversity of mycorrhiza in the rhizospheric soils of perennial grass species in the Layyah district, and their subsequent impact on mycorrhizal colonization in soil and *Cenchrus ciliaris*, and its growth and physiological characteristics.

2. Materials and Methods

2.1. Study Area

In the present study, the diversity of mycorrhiza was studied in perennial grass species from District Layyah (Thind Kalan Nashab and Chak no 122/T.D.A.), Punjab, Pakistan between 2011–2013. The study area is located between longitude 30°58′0″ N and latitude 70°56′0″ E, with an altitude of 143 m above sea level [32]. Thind Kalan Nashab and Chak no 122/T.D.A. from District Layyah is situated about 280 km from The Islamia University, Bahawalpur. The study area is located along the bank of the Indus River. Layyah derives its name from a wild short stature shrub, commonly known as Layyah. The area of the district is naturally divided into the Nasheb area, Thal, and Sandy Thal desert. The climate of Layyah is arid, where summer is extremely hot while winter is cold. The weather is dry all year round, especially in Thal areas. The other parts of the district that are flooded from the Indus River or irrigated via inundation canals are comparatively less dry. Flood season and inundation by river results in abundant moisture on the ground, as well as in the air. The moisture reaches its maximum during the inundation period (August and September). The distribution and incidence of rainfall are quite regular and go along the seasons. The average rainfall does not exceed 18.25 cm, of which main downpours are experienced during the summer months. In summer, the temperature may rise to 51 °C. Dust storms are common during May, June, and July. The area is rich in diverse vegetation cover due to the local irrigation system. Shisham (*Dalbergia sissoo* L.) is the most common tree found in almost every part of the district. The arid climate of the area gives rise mainly to the xerophytic type of shrubs and herbs. The vegetation of the area is mostly of the perennial type, where plants usually blossom during the monsoon rains. The annual herbs and grasses have a shallow-branched root system.

2.2. Rhizospheric Soil Sampling

For the collection of rhizospheric soil of perennial grasses (*Saccharum spontaneum* L., *Saccharum bengalense* Retz., *Setaria verticillata*, *Cymbopogon jwarancusa* (Jones) Schult., and *Typha angustata*), the roots were gently taken out and shaken. The soil adhering to the roots was washed with sterile distilled water to remove the loosely bound soil with the roots. After that, the soil adhering to the roots was gently removed with a sterilized scissor. The wet sieving and decanting methods were used to extract spores from each collected rhizospheric soil sample [33]. The number of spores in each soil sample was immediately counted using a Zoom Stereomicroscope (DigiStar-2, Labomed, USA) and the number of spores per 100 g of soil was recorded.

Standard procedures were followed for soil physicochemical analysis. Due to its sandy texture, the soil was suspended in distilled water in a 1:2 (w/v) ratio for pH and electrical conductivity (EC), and pH and EC were measured using pH (HACH 8190) and EC meters (HACH-44600), respectively. For carbonates, bicarbonates, calcium, and magnesium, the titration method was used. For nutrient analysis, available nitrogen was determined through the Kjeldahl method, phosphorus through the bicarbonate method [34], and potassium by using a flame photometer. Organic matter was determined using the potassium dichromate method [35].

2.3. Pot Experiment

To investigate the impact of mycorrhizal inoculation on the growth and root colonization of *Cenchrus ciliaris*, a trap culture was conducted. The soil collected during the sampling was ground, sieved (2 mm), and autoclaved for 20 min at 121 °C. About 10 kg

of soil was put in each plastic pot with a height and diameter of 30 × 20 cm. A large number of hyphae and spores collected from different rhizospheric soils were cultured on MS medium, and then the mycelium suspension containing 4000 spores mL^{-1} was prepared. For inoculation of soil with mycorrhiza, the spores (10 g or ± 50 AMF spores) [36] were placed immediately under the seedlings [37]. Six treatments were comprised of control and mycorrhizal inoculation with spores of different species collected from different rhizospheric soils (R-S) viz. control, R-S_1 (*Typha angustata*), R-S_2 (*Cymbopogon jwarancusa*, R-S_3 (*Saccharum spontaneum*), R-S_4 (*Setaria verticillata*), and, R-S_5 (*Saccharum bengalense*). In the control treatment, only autoclaved soil was used as the growth medium for *Cenchrus ciliaris*. The treatments were arranged in a completely randomized design in triplicate. During the present investigation, *Cenchrus ciliaris* was selected as a test plant due to its nutritional value and adaptability under hot desert conditions. The seeds of *Cenchrus ciliaris* were surface sterilized with $HgCl_2$ for 2–3 min and washed 3–4 times with sterilized distilled water. The sterilized seeds were pre-germinated in Petri dishes containing wet filter paper with sterilized distilled water and kept in dark. In each pot, four uniform seedlings were transplanted. The pots were irrigated with tap water daily. After three weeks of transplantation, the harvesting of *Cenchrus ciliaris* was completed.

For growth parameters, standard procedures were followed. For example, the plant height was measured by selecting the tiller with the maximum height from each pot. The selected plants were also used for measuring stem diameter with the help of the Vernier caliper. For the number of tillers $plant^{-1}$, all the plants of each pot were explored and the average was calculated. The plant's average number of leaves was computed similarly by dividing the total number of leaves by the number of tillers in each pot. The average leaf area was estimated using the following formula, which involved measuring the average leaf length and width in each pot.

$$\text{Average leaf area } (\text{cm})^2 = \text{average leaf length } (\text{cm}) \times \text{average leaf width } (\text{cm}) \times 1.75$$

$$\text{total leaf area pot}^{-1} \ (\text{cm})^2 = \text{average leaf area } (\text{cm})^2 \times \text{average no. of leaves plant}^{-1} \times \text{no. of tillers pot}^{-1}$$

For fresh biomass, the harvested plants from each pot were weighed using a digital balance (BL-320 H, Shimadzu Corporation, Tokyo, Japan). For dry weight, the plant samples were put into an oven at 65 °C for 48 h or until a constant weight was obtained. For chlorophyll contents, a portable absorbance-based dual-wavelength chlorophyll meter (SPAD-502; Minolta Corporation, Ltd., Osaka Japan) was used. Leaves from the canopy of each plant were randomly selected for the measurement of chlorophyll contents.

2.4. Root Colonization with Mycorrhiza

After harvesting the plants, the root samples in the pots were gently taken out of the soil. The root samples with intact epidermis were selected and washed carefully under running or tap water, avoiding serious damage to the epidermis, preserved in FAA (Formaldehyde: Acetic acid: Alcohol 5:5:90 *v*/*v*) solution, and kept at 4 °C until analyzed for mycorrhizal colonization. For mycorrhization, the roots were cleared, washed with KOH (10% *w*/*v*), and autoclaved at 121 °C for 20 min. After taking cooled samples from the autoclave, the root samples were treated with HCl (01 N) for five minutes and stained with trypan blue (0.05%) in lactophenol and left overnight [38].

The data regarding the frequency of mycorrhizal colonization were estimated via the glass slide method by randomly placing 300 stained root fragments (01 cm in length) on a glass slide with lactophenol. The glass slide was covered with a glass coverslip to avoid the formation of air bubbles. While quantifying mycorrhizal roots, a segment was considered mycorrhizal when any structures (such as hyphae, vesicles, or arbuscules) were observed. Biermann and Lindermann's method [39] was used to calculate the infection percentage. Three hundred root segments of each plant per sample were examined under the compound microscope (Olympus Digi 2) at the magnification of 10× and 40×. The data for types of AMF structures, such as arbuscules, vesicles, intra, and extra-radical hyphae,

were collected from each slide. The AMF colonization was estimated using the following parameters.

The percentage of hyphae/vesicles/spores/arbuscules in mycorrhizal parts of root fragments was calculated using the following formula:

$$\text{Hyphae/Vesicles/Spores/Arbuscules (\%)} = \frac{\text{No. of fragments containing hyphae/vesicles/spores/arbuscules}}{\text{Total no. of root fragments}} \times 100$$

For dark septate endophytic (DSE) and ectomycorrhizal colonization (ECM), the following formula was used:

$$\text{DSE/ECM (\%)} = \frac{\text{No. of fragments containing ectomycorrhiza/dark septate mycorrhiza}}{\text{Total no. of root fragments}} \times 100$$

For extraction of spores from the soil after harvesting *Cenchrus ciliaris*, the wet sieving and decanting method, as mentioned earlier, was adopted. The percentage, frequency, and relative frequency of spores were calculated using the following formula:

$$\text{Spores (\%)} = \frac{\text{No. of spores of one isotype}}{\text{Total no. of spores per 100 g soil}} \times 100$$

$$\text{Sporesfrequency} = \frac{\text{No. of sites containing spores}}{\text{Total number of sites}} \times 100$$

$$\text{Relativefrequency} = \frac{\text{Frequency of spores on one site}}{\text{Frequency of spores on all site}} \times 100$$

2.5. Identification of Spores

The taxonomic identification of spores was done using Morton's method [40]. The color, size, and wall structure of the spores were used in their identification. The spores or species were identified using Pérez and Schenck's [41] and Hall and Fish's keys [42].

2.6. Simpson's Diversity Index

Simpson's diversity index was calculated using the equation below, as described by Simpson [43]:

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

where n is the total number of individuals of a particular mycorrhizal species and N is the total number of individuals of all species

2.7. Statistical Analysis

The data collected during the present research work were subjected to analysis of variance using Statistix 8.1 and treatment means were compared using the least significant difference (LSD) at $p < 0.05$ [44]. GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) was used to calculate the mean values with standard deviation and for the graphical representation of data.

3. Results

3.1. Mycorrhizal Colonization

The root samples in the form of root fragments collected from five rhizospheric soils of different grasses [R-S_1 (*Saccharum spontaneum*), R-S_2 (*Saccharum bengalense*), R-S_3 (*Setaria verticillata*), R-S_4 (*Cymbopogon jwarancusa*) and R-S_5 (*Typha angustata*)] were observed under a compound light microscope to observe the percent root colonization via mycorrhiza (Figures 1 and 2a–f). Soil texture and plant species of the rhizospheric soils had a significant relation with the mycorrhizal colonization in different root fragments collected from different rhizospheric soils (Table 1 and Figure 2).

Table 1. Physicochemical properties of collected rhizospheric soil samples.

Physicochemical Properties	R-S_1 (*Typha angustata*)	R-S_2 (*Cymbopogon jwarancusa*)	R-S_3 (*Saccharum spontaneum*)	R-S_4 (*Setaria verticillata*)	R-S_5 (*Saccharum bengalense*)
EC (dS m^{-1})	1.8	3.1	3.7	3.4	2.0
pH	7.9	8.2	8.2	7.9	7.4
Total N (%)	0.38	0.36	0.34	0.36	0.38
Available P (mg kg^{-1})	1.84	1.74	1.66	1.72	1.81
K (mg kg^{-1})	170	240	280	310	320
Ca + Mg (meq L^{-1})	2.16	1.25	2.26	2.12	1.12
CO_3^{2-} (meq L^{-1})	0.52	0.48	1.4	0.72	0.4
HCO_3^{-1} (meq L^{-1})	1.99	0.56	0.16	0.92	0.7
Organic matter (%)	0.32	0.35	0.36	0.39	0.40
Texture	Sandy	Sandy	Sandy	Loamy sand	Sandy loam

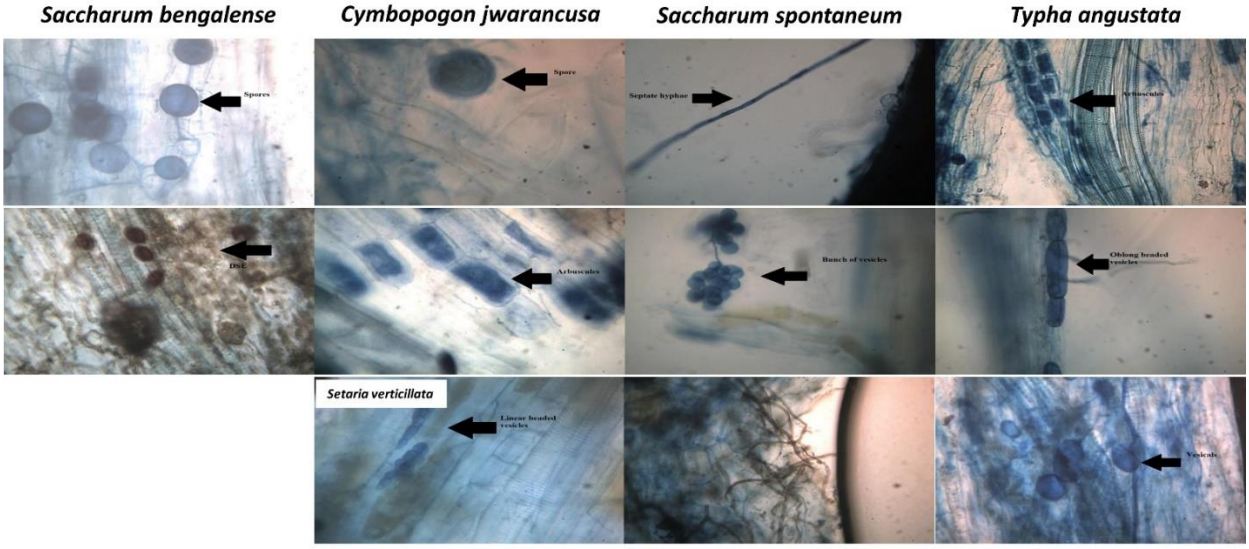

Figure 1. Hyphae, vesicles, spores, arbuscules, DSE, and ECM observed in the soil collected from the rhizospheric samples of different grasses.

As clear from Figure 2a, the maximum hyphal colonization (59.8%) was observed in the case of R-S_5, followed by R-S_4 (57.7%), R-S_1 (52.8%), and R-S_2 (52.0%). The minimum hyphal colonization was observed in the case of R-S_3 (48.7%), i.e., the rhizospheric soil collected from the roots of *Saccharum spontaneum*. The maximum hyphal colonization in the case of R-S_5 was 22.8% more than that observed in the case of R-S_3. The hyphal colonization in the case of R-S_5 was non-significantly ($p < 0.05$) different from R-S_4. In the case of vesicle colonization, the R-S_5 showed the maximum value, i.e., 12% and it was 4.3 times higher, as compared to that observed in the case of R-S_3 (Figure 2b). The root fragments collected from the rhizospheric soil of *Saccharum bengalense* (R-S_5) had the maximum spore percentage, compared to the other root fragments collected from the rhizospheric soils of different grasses, while that of the minimum was observed in the case of R-S_2, i.e., 2.3% (Figure 2c). The percentage of arbuscules colonization was the highest (11.3%) in the case of root fragments collected from the rhizospheric soil (R-S_5) of *Saccharum bengalense*, while the lowest was in the case of R-S_2, i.e., 4.0% (Figure 2d). Regarding the percent colonization of dark septate endophytic (DSE) mycorrhiza, the root fragments from the rhizospheric soil of *Saccharum bengalense* showed the maximum (12.0%), while that of the minimum (7.3%) was recorded in the case of R-S_2 (Figure 2e). In the case of ectomycorrhizal (ECM) colonization, the maximum colonization (59.8%) was observed in the case of R-S_5, followed by R-S_4 (58.0%) and R-S_2 (54.2%) (Figure 2f).

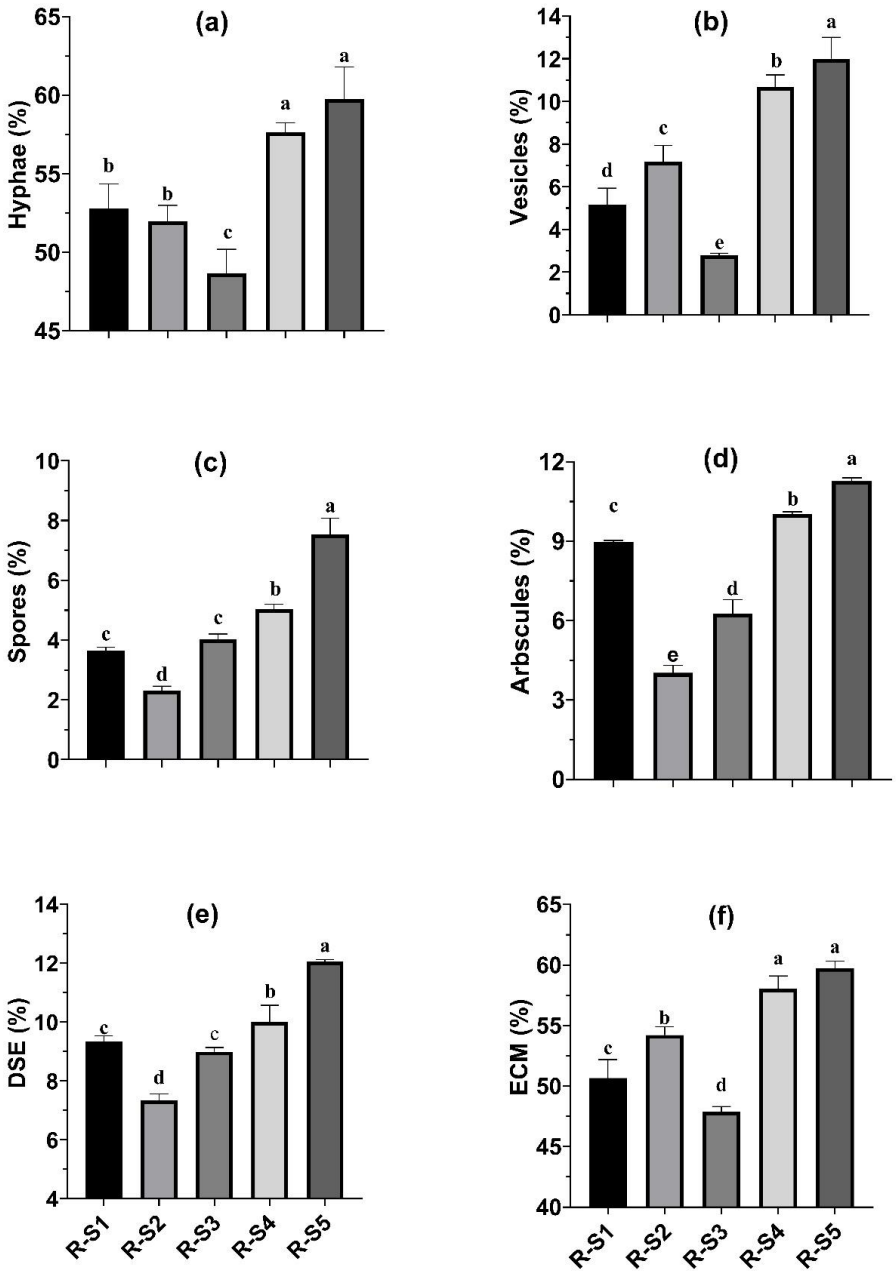

Figure 2. Hyphal (**a**), vesicles (**b**), spores (**c**), arbuscules (**d**), DSE (**e**), and ECM (**f**) colonization in the roots of *Cenchrus ciliaris* extracted from trap culture experiment grown in various rhizospheric soils from selected plants. Each column with a different letter is significantly different at $p < 0.05$. Where R-S_1 = rhizospheric soil of *Typha angustata*, R-S_2 = rhizospheric soil of *Cymbopogon jwarancusa*, R-S_3 = rhizospheric soil of *Saccharum spontaneum*, R-S_4 = rhizospheric soil of *Setaria verticillata*, R-S_5 = rhizospheric soil of *Saccharum bengalense*.

The minimum ECM colonization (47.9%) was recorded in the case of R-S_3 root fragments collected from the rhizospheric soil of *Saccharum spontaneum* and it was 24.8% less, in comparison to that observed in the case of root fragments of R-S_5.

3.2. Growth Parameters and Chlorophyll Contents

The application of rhizospheric soils of different plants in *Cenchrus ciliaris* caused a significant increase in various growth parameters recorded (Figure 3a–f). In the case of plant height, the maximum (21.55 cm) was recorded with the application of rhizospheric

soil from *Saccharum bengalense* (R-S_5), while the minimum (18.03 cm) was observed with the application of rhizospheric soil from *Cymbopogon jwarancusa* (R-S_2) and it was 19.5% more compared to that observed with R-S_2 (Figure 3a). The number of tillers plant^{-1}, the fresh weight of shoot and root, and the dry weight of shoot and root all showed a similar trend (Figure 3b–f). With the application of rhizospheric soil from *Saccharum bengalense* (R-S_5), the maximum number of tillers plant^{-1} (8.24), fresh weight of shoot (22.96 g) and root (9.38 g), and dry weight of shoot (8.63 g) and root (2.56 g) in *Cenchrus ciliaris* were observed.

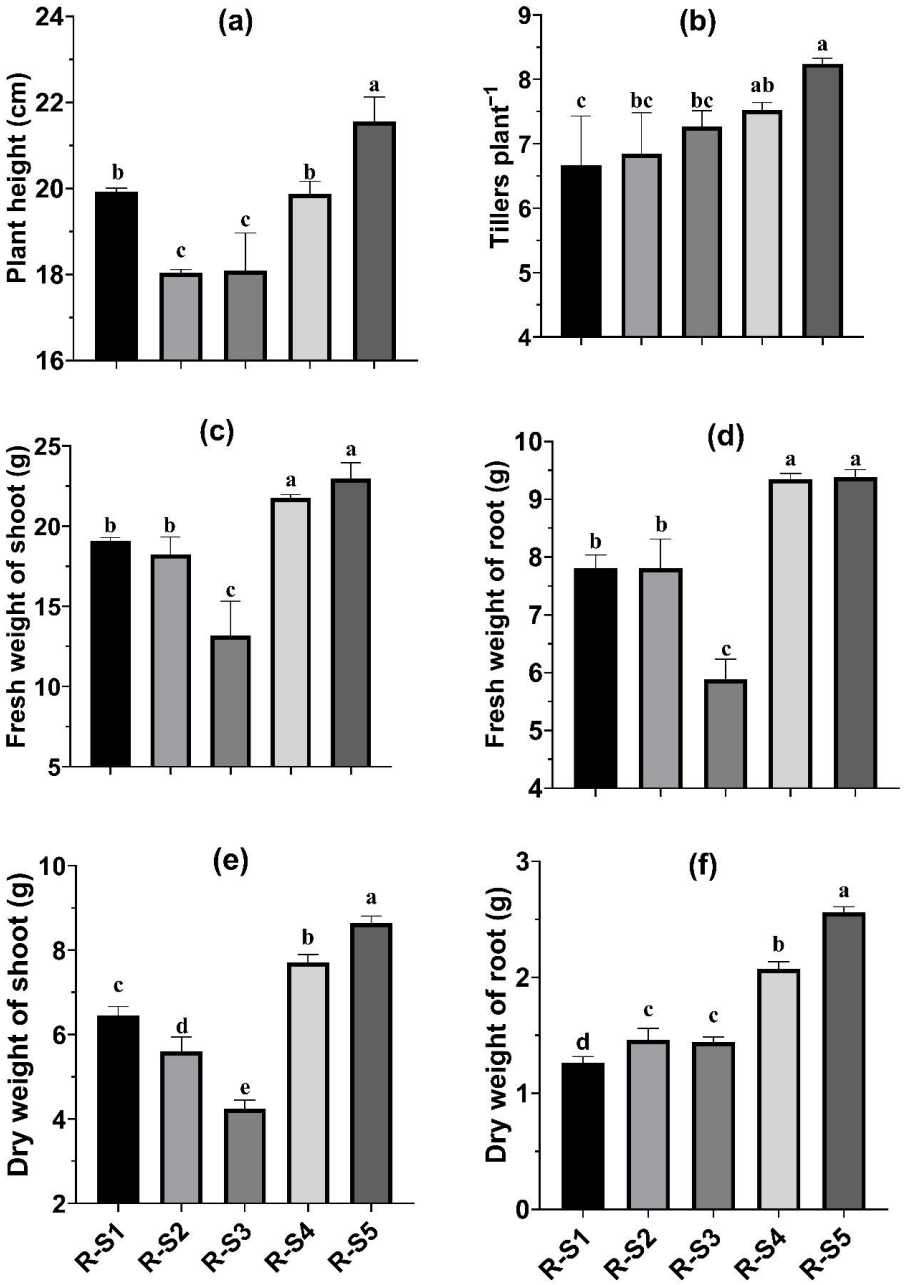

Figure 3. Growth parameters, i.e., plant height (**a**), tillers plant^{-1} (**b**), fresh weight of shoot (**c**), fresh weight of root (**d**), dry weight of shoot (**e**), dry weight of root (**f**) of *Cenchrus ciliaris*, as affected by the application of rhizospheric soils from selected plants. Each column with a different letter is significantly different at $p < 0.05$. Where R-S_1 = rhizospheric soil of *Typha angustata*, R-S_2 = rhizospheric soil of *Cymbopogon jwarancusa*, R-S_3 = rhizospheric soil of *Saccharum spontaneum*, R-S_4 = rhizospheric soil of *Setaria verticillata*, R-S_5 = rhizospheric soil of *Saccharum bengalense*.

The maximum values of the number of leaves plant^{-1} (13.21), leaf area (13.31 cm^2), and chlorophyll contents (39.46 g plant^{-1}) were observed with the application of rhizospheric soil obtained from *Saccharum bengalense* (R-S_5), which were 17.6, 108, and 29.4% higher than those observed with the application of rhizospheric soil obtained from *Saccharum spontaneum* (R-S_3) (Figure 4a–c).

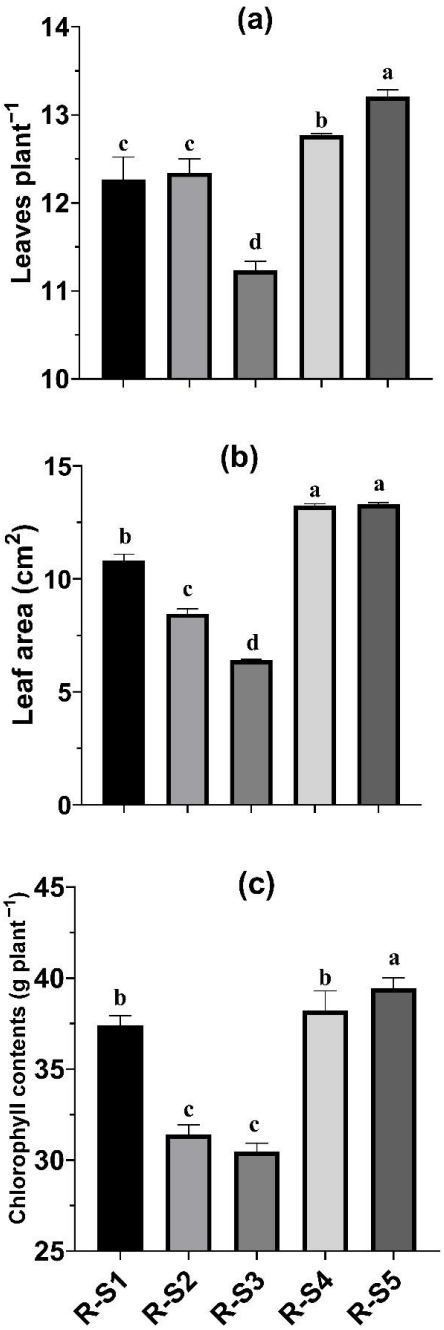

Figure 4. Physiological parameters, i.e., number of leaves plant^{-1} (**a**), leaf area (**b**), and chlorophyll contents (**c**) of *Cenchrus ciliaris*, as affected by the application of rhizospheric soils from selected plants. Each column with a different letter is significantly different at $p < 0.05$. Where R-S_1 = rhizospheric soil of *Typha angustata*, R-S_2 = rhizospheric soil of *Cymbopogon jwarancusa*, R-S_3 = rhizospheric soil of *Saccharum spontaneum*, R-S_4 = rhizospheric soil of *Setaria verticillata*, R-S_5 = rhizospheric soil of *Saccharum bengalense*.

3.3. Mycorrhizal Spore Diversity in Rhizospheric Soils

Mycorrhizal spore diversity in rhizospheric soil collected from different grasses is shown in Table 2. It is clear from the data presented in Table 2 that spores belonging to *Glomus glomerculatum* and *Glomus pastulatum* were the highest in number while that of the *Acaulospora* species were present in the lowest number in the case of the soil (R-S_3) collected from the rhizosphere of *Saccharum spontaneum*. The maximum relative frequency (12.5) was recorded in the case of *Glomus glomerculatum* and *Glomus pastulatum* while the minimum (2.41) was observed in the case of *Acaulospora* species. The absolute frequency ranged from 0.33 to 0.83 (Table 2). In the case of R-S_5, the maximum spores with maximum relative frequency (12.5) and species richness (n = 5) belonged to *Glomus mosseae* and *Glomus tortuosum*, while that of the minimum (2.23 and n = 2) in the case of *Acaulospora bireticulata*, respectively.

Table 2. Diversity of mycorrhizal spores in rhizospheric soils of different grass species.

R-S_1 (*Typha angustata*)				
AMF Species	**Absolute Frequency**	**Relative Frequency**	**Species Richness (n)**	**n(n-1)**
Glomus Constrictum (Trappe)	0.16	2.41	2	2
Glomus clariodium (Schenck and Smith)	0.83	12.50	5	20
Glomus glomerculatum (Sieverd)	0.16	2.41	2	2
Glomus etunicatum (W.N.Becker and Gred)	0.16	2.41	2	2
Glomus clarum (Nicolson and Schenk)	0.83	12.50	5	20
Glomus mosseae (Nicol. and Gerd.) Gerdemann and Trappe.	0.16	2.41	2	2
Acaulospora scrobiculata (Trappe)	0.33	4.98	2	2
Glomus maculosum (Walker and Vestberg)	0.33	4.98	2	2
Total			22	52
Simpson's Index of Diversity (D)			**0.8874**	
R-S_2 (*Cymbopogon jwarancusa*)				
Acaulospora scrobiculata (Trappe)	0.83	12.50	5	20
Scutellospora nigra (Red head) Walker and Sanders	0.16	2.41	2	2
Glomus glomerculatum (Sieverd)	0.16	2.41	2	2
Glomus mosseae (Nicol. and Gerd.) Gerdemann and Trappe.	0.83	12.50	5	20
Glomus aggregatum (N.C.Schenchea G.S.Sm.emend, Koske)	0.83	12.50	5	20
Glomus constrictum (Trappe)	0.16	2.41	2	2
Glomus etunicatum (W.N.Becker and Gred)	0.16	2.41	2	2
Glomus pastulatum (Trappe)	0.83	12.50	5	20
Glomus clarioidium (Schenck and Smith)	0.83	12.50	5	20
Glomus maculosum (Walker and Vestberg)	0.16	2.41	2	2
Total			35	110
Simpson's Index of Diversity (D)			**0.9076**	
R-S_3 (*Saccharum spontaneum*)				
Acaulospora spp.	0.16	2.41	2	2
Glomus glomerculatum (sieverd)	0.83	12.5	5	20
Glomus microaggregatum	0.33	4.98	2	2
Glomus pastulatum (Koske, Friese, C. Walker and Dalpe)	0.83	12.5	5	20
Glomus diaphanum (J.B.Morton and C.Walker)	0.33	4.98	2	2
Total			16	46
Simpson's Index of Diversity (D)			**0.8083**	
R-S_4 (*Setaria verticillata*)				
Acaulospora scrobiculata (Trappe)	0.83	12.50	5	20
Glomus fasciculatum (Gred and Trappe emend. Walker and Koske)	0.16	2.41	2	2
Glomus mosseae (Nicol. and Gred.) Gerdemann and Trappe.	0.16	2.41	2	2
Glomus aggregatum (N.C.Schenchea G.S.Sm.emend, Koske)	0.16	2.41	2	2
Glomus maculosum (Walker and Vestberg)	0.33	4.98	2	2

Table 2. *Cont.*

R-S_4 (*Setaria verticillata*)				
AMF Species	**Absolute Frequency**	**Relative Frequency**	**Species Richness (n)**	**n(n-1)**
Gigaspora (Becker and Hall)	0.33	4.98	2	2
Glomus pastulatum (Koske, Friese, C. Walker and Dalpe)	0.83	12.50	5	20
Glomus etunicatum (W.N.Becker and Gred)	0.33	4.98	2	2
Glomus constrictum (Trappe)	0.16	2.41	2	2
Glomus tortuosum (N.C. Schenck and G.S.SM)	0.16	2.41	2	2
Total			26	56
Simpson's Index of Diversity (D)		**0.9138**		
R-S_5 (*Saccharum bengalense*)				
Glomus fasciculatum (Gred and Trappe emend. Walker and Koske)	0.33	4.98	2	2
Acaulospora bireticulata (F. M. Rothwell and Trappe)	0.4	2.23	2	2
Glomus maculosum (Walker and Vestberg)	0.33	4.98	2	2
Glomus mosseae (Nicol.andGred.) Gerdemann and Trappe	0.83	12.5	5	20
Acaulospora scrobiculata (Trappe)	0.16	2.41	2	2
Glomus diaphanum (J.B.Morton and C.Walker)	0.33	4.98	2	2
Glomus clarum (Nicolson and Schenck)	0.16	2.41	2	2
Glomus aggregatum (N.C.Schenchea G.S.Sm.emend, Koske)	0.16	2.41	2	2
Glomus geosporum (Nicol. and Gerd.) Walker	0.16	2.41	2	2
Glomus tortuosum (N.C. Schenck and G.S.SM)	0.83	12.50	5	20
Glomus constrictum (Trappe)	0.16	2.41	2	2
Glomus microaggregatum	0.16	2.41	2	2
Total			30	60
Simpson's Index of Diversity (D)		**0.9310**		

Similarly, the spores belonging to *Glomus etunicatum* and *Acaculospora scrobiculata* were present in the largest number in the case of the soil collected from the rhizosphere of *Setaria verticillata* (R-S_4), while the lowest number of species belonged to *Glomus fasiculatum*, *Glomus mosseae*, *Glomus aggregatum*, and *Glomus constrictum* species. In R-S_4, *Acaculospora scorbiculata* was observed with the maximum relative frequency, i.e., 12.5 while that of the minimum relative frequency, i.e., 2.41 was recorded in the case of *Glomus constrictum*. The maximum relative frequency (12.5) in the case of R-S_2 was recorded in the case of *Acaulospora scrobiculata*, *Glomus mosseae*, *Glomus aggregatum*, *Glomus pastulatum*, and *Glomus clarioidium*, while that of the minimum (2.41) was observed in the case of *Scutellospora nigra*, *Glomus glomerculatum*, *Glomus constrictum*, *Glomus etunicatum*, and *Glomus maculosum*. *Glomus clariodium* and *Glomus clarum* spore species had the maximum relative frequency (12.5) in the soil collected from the rhizosphere of *Typha angustata* (R-S_1), while that of the minimum (2.41) was recorded in the case of *Glomus mosseae*, *Glomus constrictum*, *Glomus etunicatum*, and *Glomus aggregatum*. The absolute frequency ranged from 0.33 to 0.83 (Table 2). In the case of Simpson's index of diversity, the maximum (0.9310) was observed in the case of R-S_5, followed by R-S_4 (0.9138), R-S_2 (0.9076), and R-S_1 (0.8874), and the minimum was observed in the case of R-S_3 (0.8083).

3.4. Mycorrhizal Spore Diversity in Rhizospheric Soil of Cenchrus ciliaris

After harvesting the *Cenchrus ciliaris*, the rhizospheric soil was analyzed regarding mycorrhizal spore diversity (Table 3). The maximum relative frequency of spores belonging to *Acaulospora Scrobiculata* was 9.96, while that of the minimum, i.e., 1.61 belonged to *Acaulospora* spp. and *Scutellospora nigra*. The absolute frequency ranged from 0.16 to 0.83. A Simpson's index of diversity, i.e., 0.9586 was calculated from the spore profile of the soil collected from the rhizosphere of *Cenchrus ciliaris*.

Table 3. Diversity of mycorrhizal spores in rhizospheric soil of trap cultured with *Cenchrus ciliaris* after harvesting.

AMF Species	*Saccharum spontaneum*	*Saccharum bengalense*	*Setaria verticillata*	*Cymbopogon jwarancusa*	*Typha angustata*	Absolute Frequency	Relative Frequency	Species Richness (n)	n(n-1)
Acaulospora bireticulata (F.M. Rothwell and Trappe)	-	+	-	-	-	0.16	2.41	2	2
Acaulospora spp.	+	-	-	-	-	0.20	1.61	1	0
Acaulospora scrobiculata (Trappe)	-	+	+	+	-	0.66	9.96	4	12
Scutellospora nigra (Red head) Walker and Sanders	-	-	-	+	-	0.20	1.61	1	0
Glomus fasciculatum (Gred and Trappe emend. Walker and Koske)	-	+	+	-	-	0.33	4.98	2	2
Glomus glomerculatum (Sieverd)	+	-	-	+	+	0.50	7.55	3	6
Glomus mosseae (Nicol. and Gerd.) Gerdemann and Trappe.	-	-	+	+	+	0.40	3.23	2	2
Glomus monosporum (Gerdemann and Trappe)	+	+	-	-	-	0.40	3.23	2	2
Glomus aggregatum (N.C. Schenchea G.S.Sm.emend, Koske)	-	+	+	+	-	0.16	2.41	2	2
Glomus clarum (Nicolson and Schenk)	-	+	-	-	+	0.60	4.84	3	6
Glomus diaphanum (W.N. Becker and Gred)	+	+	-	-	-	0.33	4.98	2	2
Glomus maculosum (Walker and Vestberg)	-	+	+	+	+	0.83	12.5	5	20
Gigaspora (Becker and Hall)	-	-	+	+	+	0.33	4.98	2	2
Glomus constrictum (Trappe)	-	+	+	+	+	0.80	6.45	4	12
Glomus pastulatum (Trappe)	+	+	+	-	-	0.50	7.55	3	6
Glomus etunicatum (W.N. Becker and Gred)	-	-	+	+	+	0.40	3.23	2	2
Glomus clarioidium (Schenck and Smith)	-	-	-	+	+	0.33	4.98	2	2
Glomus microaggregatum	-	+	-	-	-	0.16	2.41	1	0
Glomus tortuosum (N.C. Schenck and G.S.SM)	-	+	+	-	-	0.33	4.98	2	2
Total						7.62		45	82
Simpson's Index of Diversity (D) = 0.9586									

3.5. Correlation among Growth Parameters and Diversity Characteristics of Mycorrhizal Fungi

Table 4 shows the correlation among growth parameters, such as dry weight of root, dry weight of shoot, fresh weight of root, fresh weight of shoot, leaf area, no. of leaves, plant height, and no. of tillers and diversity characteristics, i.e., Simpson's diversity index and species richness of mycorrhizal fungi. Simpson's diversity index had a significant relationship ($p < 0.05$) with growth parameters, such as the fresh weight of the root, the fresh weight of the shoot, and the no. of leaves. Similarly, the growth parameters, such as chlorophyll contents, dry weight of root and shoot, fresh weight of root and shoot, leaf area, no. of tillers, plant height, and no. of leaves, had a significant relationship ($p < 0.05$) among themselves.

Table 4. Correlation among growth parameters and diversity characteristics of mycorrhizal fungi.

	Chloro	D	DWR	DWS	FWR	FWS	LA	Leaves	PH	Tillers
D	0.7039									
DWR	0.6544	0.5969								
DWS	0.9326 *	0.8588	0.8271							
FWR	0.8478	0.9369 *	0.7538	0.9601 **						
FWS	0.8844 *	0.9447 *	0.7374	0.9759 **	0.9901 **					
LA	0.9613 **	0.8245	0.7417	0.9782 **	0.9555 *	0.9618 **				
Leaves	0.823	0.9651 **	0.762	0.9583 *	0.9758 **	0.9885 **	0.9153 *			
PH	0.9446 *	0.6412	0.762	0.9162 *	0.7707	0.8278	0.8833 *	0.8013		
Tillers	0.502	0.3378	0.9537 *	0.6524	0.5266	0.5142	0.551	0.5443	0.6729	
n	0.1784	0.8198	0.3587	0.4610	0.6152	0.6041	0.3693	0.6927	0.1730	0.1393

Where Chloro = Chlorophyll contents; D = Simpson's Diversity Index; DWR = Dry weight of root; DWS = Dry weight of shoot; FWR = Fresh weight of root; FWS = Fresh weight of shoot; LA = Leaf area; Leaves = No. of leaves; PH = Plant height; Tillers = no. of tillers; and n = Species richness. * = significant at $p < 0.05$, ** = significant at $p < 0.01$.

4. Discussion

The roots of higher plants live in symbiosis with mycorrhizal fungi [45]. AMF are obligate biotrophic fungi, found in the roots of plants with hyphae, vesicles, spores, arbuscules, dark septate endophytes (DSE), and ectomycorrhiza (ECM), and they can improve the mineral nutrition of host plants and, in turn, take carbohydrates from the host plant [46]. Earlier, a positive correlation between AMF spore density and root colonization was reported [47,48]. The density of viable AMF spores recovered from rhizosphere soil samples collected from the field and subsequent pot cultures ranged between 16 and 45 spores in 10 g^{-1} soil for the plants studied in this study (Tables 2 and 3). The spore density was low, which is typical of arid and semi-arid environments [49]. Panwar and Tarafdar [50] attribute these differences to the length of the growing season and the type of tree root systems, which make the rhizosphere more conducive to spore propagation and AMF colonization [51]. Soil features have been shown to influence the structure and composition of AMF communities, and they have recently been identified as one of the essential variables in the formation of AMF communities [52]. Cofré et al. [53] reported variation in AMF species richness and relatively lower AMF colonization rates in different types of soil, which is consistent with our results.

Manoharan et al. [54] discovered that the genus *Funneliformis* had a higher mean relative abundance in agricultural soils, whereas *Septoglomus* was more abundant in permanent pasture grasslands, and *Rhizophagus* was more plentiful in permanent pastures and fields. Diversispora and Clareidoglomas were also more common in soils during organic farming, corroborating our findings. The richness and diversity of AMF communities have been shown to vary with environmental factors (climate and soil conditions) and spatial distance [55,56]. According to Zhu et al. [55], *Ambispora*, *Archaeospora*, *Claroideoglomus*, *Gigaspora*, *Glomus*, *Paraglomus*, and *Scutellospora* were dominant at the genus level and accounted for over 99% of the recovered sequence reads. AM fungal endophytes, i.e., DSE

were found in all of the soil samples in our investigation, but the percentages varied. The brown fungus that was not stained by the stain represented the non-mycorrhizal infection.

AMF colonize plant roots differently, resulting in a variety of effects on plant growth, biomass allocation, and photosynthesis [8,16]. AMF colonization was reduced in soils with high phosphate concentrations in previous research. The available phosphorus contents in the soil through the application of chemical fertilizers were the major limiting factor influencing the colonization rates in the different soil types utilized in the current study [57].

Similarly, growth and physiological parameters were also enhanced with the application of rhizospheric soils containing AMF in the present study. According to Liang et al. [58], the diversity of different fungal species exerts a positive impact on the distribution of nitrogen and phosphorus and ultimately on plant growth and productivity. The increase in the availability of essential nutrients might have increased various growth and physiological parameters of *Cenchrus ciliaris* in the present study. Therefore, AMF diversity could be a significant contribution toward enhanced plant performance and sustainability in ecosystems [55]. Moreover, growth parameters are also affected by the fungal association in the root zone of plants fungal colonization, aiding the host plant in maintaining ionic balance by enhancing and/or selective uptake of nutrients [59]. Studies have demonstrated that arbuscules mycorrhizal connections are helpful for the plants growing under various Indian semi-arid settings [60].

Mycorrhizal symbiosis is the most common and ubiquitous plant–microbe interaction, and it is important for plant phosphorus supply and plant function in a variety of ways [1,18]. Improved nutrient uptake (mostly phosphorus), protection of roots against diseases, and easing of water stress are some of the immediate benefits that plants may reap [19,20]. Plants may improve their competitive ability because of these immediate benefits. Plant height, fresh and dry weight of shoot, and root of *Cenchrus ciliaris* increased the most in rhizospheric soils of *Saccharum bengalense*, compared to other treatments, possibly due to the favorable response of mycorrhizal inoculation under the moderate fertility state of the soil [2,3]. This is consistent with previous findings, which indicated that mycorrhizal inoculation raised the weight of plant roots and shoots substantially. These findings back up previous research that found that mycorrhizal inoculation benefits plants [18,21]. According to one interpretation of our findings, the soil treatments would have an impact on the indigenous AM community, which would then have a good impact on the plant community. Alloush et al. [61], Zaidi et al. [62], and Akhtar and Siddiqui [63] all reported on the impact of AMF on nutrient uptake in chickpeas. *A. porrum* had previously been shown to have a good influence on growth characteristics, such as root length, root number, and branching [10]. Similarly, Bago and Becard [8] and Huo et al. [16] found that inoculating mycorrhizal fungus enhanced branching quantity.

The leaf area of *Cenchrus ciliaris* was greatly enlarged in the present study after the application of rhizospheric soil from *Saccharum bengalense*. These findings are consistent with Chaudhry et al. [31], who discovered that when grasses were inoculated with VA endophytes, i.e., DSE, the biomass of the grasses rose considerably. Chaudhry et al. [64] studied morphological mycorrhizal diversity in two aromatic types of grasses (*C. jawaruncusa* and *V. zizinoides*). In results, no arbuscules were observed. Arbuscules are highly branched tree-like structures, which transfer phosphorus and other nutrients to plants [61–63]. However, in the present, arbuscules were observed, which might be due to the difference in the geographic location of the area from which the samples were collected. In the present study, AMF colonization was highly variable [65].

Diversity studies showed that only a few fungal spore morphotypes, i.e., *A. bireticulata* and *Glomus* species were consistently detected in all rhizospheric soils of the target plant species. These results are in line with other studies, which found similar species under arid and semi-arid environments [66,67]. The dominance of *Glomus* sp. might be attributed to its different temperature preferences [65]. Despite the rigorous research work presented, investigations in the future can elucidate the role of rhizospheric fungi in improving the

growth and yield of plants under multiple abiotic stresses, such as drought and salinity, which was beyond the scope of the present study.

5. Conclusions

The present study confirmed that mycorrhizal fungi in the rhizospheric soils of different plants had a positive impact on the growth parameters of native grass (*Cenchrus ciliaris*) of district Layyah. From the analysis of rhizospheric soils from different plants, it was revealed that the maximum hyphal, vesicles, arbuscules, dark septate endophytic and ectomycorrhizal colonization, and spore percentage were observed in the case of R-S_5, i.e., rhizospheric soil collected from *Saccharum bengalense*. However, the maximum (0.9310) Simpson's index of diversity was observed in the case of R-S_2, i.e., rhizospheric soil collected from *Saccharum bengalense*. The application of rhizospheric soil collected from *Saccharum bengalense* (R-S_5) caused the maximum increase in growth and physiological parameters of *Cenchrus ciliaris*. In conclusion, the inoculation of mycorrhizal fungi significantly improved the mycorrhizal characteristics of *Cenchrus ciliaris* and its rhizospheric soil and ultimately enhanced the growth and physiological parameters of *Cenchrus ciliaris*. The rhizospheric fungi tested in the present study had great potential and, therefore, could be tested and validated under arid land conditions to enhance the growth and productivity of different crops facing simultaneous abiotic stresses of drought and salinity.

Author Contributions: Conceptualization, S.T. and M.S.C.; Data curation, S.T., A.D., I.H., N.U. and Q.M.; Formal analysis, A.D., I.H., A.P., N.U. and Q.M.; Funding, I.A.-A., A.E.-S.; Investigation, S.T.; Methodology, M.S.C., I.H., A.P. and Q.M.; Project administration, M.S.C., A.D., I.H. and Q.M.; Resources, S.T., M.S.C., A.D., I.H., A.P., N.U., I.A.-A., A.E.-S. and Q.M.; Software, A.D. and A.P.; Supervision, M.S.C.; Validation, S.T., M.S.C., A.D., I.H., A.P., N.U., I.A.-A., A.E.-S. and Q.M.; Visualization, S.T., M.S.C., A.D., I.H., A.P., N.U., I.A.-A., A.E.-S. and Q.M.; Writing—original draft, S.T. and N.U.; Writing—review and editing, S.T., M.S.C., A.D., I.H., A.P., N.U., I.A.-A., A.E.-S. and Q.M. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Researchers Supporting Project number (RSP-2021/298), King Saud University, Riyadh, Saudi Arabia.

Data Availability Statement: Not applicable.

Acknowledgments: The authors extend their appreciation to the Researchers Supporting Project number (RSP-2021/298), King Saud University, Riyadh, Saudi Arabia. The Department of Life Sciences, Cholistan Institute of Desert Studies, Islamia University of Bahawalpur Pakistan provided financial support for this research. The authors also acknowledge the Cholistan Institute of Desert Studies (CIDS) and all Department of Life Sciences teachers for their assistance with laboratory statistical analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Corcoz, L.; Păcurar, F.; Pop-Moldovan, V.; Vaida, I.; Stoian, V.; Vidican, R. Mycorrhizal patterns in the roots of dominant *Festuca rubra* in a high-natural-value grassland. *Plants* **2021**, *11*, 112. [CrossRef] [PubMed]
2. Jing, M.; Shi, Z.; Zhang, M.; Zhang, M.; Wang, X. Nitrogen and phosphorus of plants associated with arbuscular and ectomycorrhizas are differentially influenced by drought. *Plants* **2022**, *11*, 2429. [CrossRef] [PubMed]
3. Da Costa, L.S.; Grazziotti, P.H.; Fonseca, A.J.; dos Santos Avelar, D.C.; Rossi, M.J.; de Barros Silva, E.; da Costa, E.C.S.; Grazziotti, D.C.F.S.; Ragonezi, C. Eucalyptus field growth, and colonization of clones pre-inoculated with ectomycorrhizal fungi. *Agronomy* **2022**, *12*, 1204. [CrossRef]
4. Marupakula, S.; Mahmood, S.; Clemmensen, K.E.; Jacobson, S.; Högbom, L.; Finlay, R.D. Root-associated fungi respond more strongly than rhizosphere soil fungi to N fertilization in a boreal forest. *Sci. Total Environ.* **2021**, *766*, 142597. [CrossRef] [PubMed]
5. Vidal, C.; González, F.; Santander, C.; Pérez, R.; Gallardo, V.; Santos, C.; Aponte, H.; Ruiz, A.; Cornejo, P. Management of rhizosphere microbiota and plant production under drought stress: A comprehensive review. *Plants* **2022**, *11*, 2437. [CrossRef]
6. Khan, Y.; Shah, S.; Hui, T. The roles of arbuscular mycorrhizal fungi in influencing plant nutrients, photosynthesis, and metabolites of cereal crops—A review. *Agronomy* **2022**, *12*, 2191. [CrossRef]
7. Ahmad, S.S.; Ahmad, T.; Rashid, A. The mediation of mycorrhizae in constituting association and distribution pattern of some plants in Murree Hills and Galliyats. *Pak. J. Biol. Sci.* **2004**, *7*, 1172–1176. [CrossRef]

8. Bago, B.; Bacrad, G. Bases of the obligate biotrophy of arbuscular mycorrhizal fungi. In *Mycorrhizal Technology in Agriculture*; Gianinazzi, S., Schuvpp, H., Barea, J.M., Haselwandter, K., Eds.; Birkhauser: Basel, Switzeland, 2002; pp. 33–48.
9. Thapa, B.; Mowrer, J. Effects of carbon amendments, tillage and cover cropping on arbuscular mycorrhizal fungi association and root architecture in corn and cotton crop sequence. *Agronomy* **2022**, *12*, 2185. [CrossRef]
10. An, X.; Liu, J.; Liu, X.; Ma, C.; Zhang, Q. Optimizing phosphorus application rate and the mixed inoculation of arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria can improve the phosphatase activity and organic acid content in alfalfa soil. *Sustainability* **2022**, *14*, 11342. [CrossRef]
11. Yurkov, A.P.; Puzanskiy, R.K.; Kryukov, A.A.; Gorbunova, A.O.; Kudriashova, T.R.; Jacobi, L.M.; Kozhemyakov, A.P.; Romanyuk, D.A.; Aronova, E.B.; Avdeeva, G.S.; et al. The role of *Medicago lupulina* interaction with *rhizophagus irregularis* in the determination of root metabolome at early stages of am symbiosis. *Plants* **2022**, *11*, 2338. [CrossRef]
12. Brundrett, M.C.; Piche, Y.; Peterson, R.L. A developmental study of the early stages in vesicular-arbuscular mycorrhiza formation. *Can. J. Bot.* **1985**, *63*, 184–194. [CrossRef]
13. Marquez, L.; Redman, R.; Rodriguez, R.; Roossinck, M. A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance. *Science* **2007**, *315*, 513–515. [CrossRef]
14. Duell, E.B.; Cobb, A.B.; Wilson, G.W.T. Effects of commercial arbuscular mycorrhizal inoculants on plant productivity and intra-radical colonization in native grassland: Unintentional de-coupling of a symbiosis? *Plants* **2022**, *11*, 2276. [CrossRef]
15. Munir, N.; Hanif, M.; Abideen, Z.; Sohail, M.; El-Keblawy, A.; Radicetti, E.; Mancinelli, R.; Haider, G. Mechanisms and strategies of plant microbiome interactions to mitigate abiotic stresses. *Agronomy* **2022**, *12*, 2069. [CrossRef]
16. Huo, L.; Gao, R.; Hou, X.; Yu, X.; Yang, X. Arbuscular mycorrhizal and dark septate endophyte colonization in Artemisia roots respond differently to environmental gradients in eastern and central China. *Sci. Total Environ.* **2021**, *795*, 148808. [CrossRef]
17. Jumrani, K.; Bhatia, V.S.; Kataria, S.; Alamri, S.A.; Siddiqui, M.H.; Rastogi, A. Inoculation with arbuscular mycorrhizal fungi alleviates the adverse effects of high temperature in soybean. *Plants* **2022**, *11*, 2210. [CrossRef]
18. Mitra, D.; Djebaili, R.; Pellegrini, M.; Mahakur, B.; Sarker, A.; Chaudhary, P.; Khoshru, B.; Gallo, M.D.; Kitouni, M.; Barik, D.P.; et al. Arbuscular mycorrhizal symbiosis: Plant growth improvement and induction of resistance under stressful conditions. *J. Plant Nutr.* **2021**, *44*, 1993–2028. [CrossRef]
19. Ai, Y.-J.; Li, F.-P.; Yang, J.-Q.; Lu, S.; Gu, H.-H. Research progress and potential functions of AMF and GRSP in the ecological remediation of metal tailings. *Sustainability* **2022**, *14*, 9611. [CrossRef]
20. Säle, V.; Sieverding, E.; Oehl, F. Growth responses of three European weeds on different AMF species during early development. *Plants* **2022**, *11*, 2020. [CrossRef]
21. Oliveira, J.S.D.; Ramos, N.P.; Júnior, J.L.; Xavier, L.P.; Andrade, E.H.; Mello, A.H.; Setzer, W.N.; Da Silva, J.K.R. Secondary metabolism and plant growth of *Piper divaricatum* (*Piperaceae*) inoculated with arbuscular mycorrhizal fungi and phosphorus supplementation. *Agronomy* **2022**, *12*, 596. [CrossRef]
22. Nafady, N.A.; Sultan, R.; El-Zawahry, A.M.; Mostafa, Y.S.; Alamri, S.; Mostafa, R.G.; Hashem, M.; Hassan, E.A. Effective and promising strategy in management of tomato root-knot nematodes by *Trichoderma harzianum* and arbuscular mycorrhizae. *Agronomy* **2022**, *12*, 315. [CrossRef]
23. Gujre, N.; Soni, A.; Rangan, L.; Tsang, D.C.; Mitra, S. Sustainable improvement of soil health utilizing biochar and arbuscular mycorrhizal fungi: A review. *Environ. Pollut.* **2021**, *268*, 115549. [CrossRef] [PubMed]
24. Liu, Y.Z.; Imtiaz, M.; Ditta, A.; Rizwan, M.S.; Ashraf, M.; Mehmood, S.; Aziz, O.; Mubeen, F.; Ali, M.; Elahi, N.N.; et al. Response of growth, antioxidant enzymes and root exudates production towards As stress in *Pteris vittata* and *Astragalus sinicus* colonized by arbuscular mycorrhizal fungi. *Environ. Sci. Pollut. Res.* **2020**, *27*, 2340–2352.
25. Quraishi, M.A.A.; Khan, K.G.; Yaqoob, M.S. *Range Management in Pakistan*; Qazi Publications: Lahore, Pakistan, 1993.
26. Gohl, B. *Tropical Feeds. Feed Information Summaries and Nutritive Values*; FAO Animal Production and Health Series 12; FAO: Rome, Italy, 1981.
27. Kheyri, Z.; Moghaddam, M.; Farhadi, N. Inoculation efficiency of different mycorrhizal species on growth, nutrient uptake, and antioxidant capacity of *Calendula officinalis* L.: A comparative study. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 1160–1172. [CrossRef]
28. Qin, Y.; Zhang, W.; Feng, Z.; Feng, G.; Zhu, H.; Yao, Q. Arbuscular mycorrhizal fungus differentially regulates P mobilizing bacterial community and abundance in rhizosphere and hyphosphere. *Appl. Soil Ecol.* **2022**, *170*, 104294. [CrossRef]
29. Gerami, Z.; Lakzian, A.; Hemati, A.; Amirifar, A.; Asgari Lajayer, B.; Van Hullebusch, E.D. Effect of cadmium on sorghum root colonization by glomerular fungi and its impact on total and easily extractable glomalin production. *Environ. Sci. Pollut. Res.* **2021**, *28*, 34570–34583. [CrossRef]
30. Heydari, L.; Bayat, H.; Gregory, A.S. Investigating the effect of inoculation of chickpea with Rhizobium and mycorrhizal fungi (*Funneliformis mosseae*) on soil mechanical and physical behavior. *Geoderma* **2021**, *385*, 114860. [CrossRef]
31. Chaudhry, M.S.; Nasim, F.H.; Khan, A.G. Mycorrhizas in the perennial grasses of Cholistan desert, Pakistan. *Int. J. Bot.* **2006**, *2*, 210–218. [CrossRef]
32. Ullah, A.; Ahmad, I.; ur Rahman, M.H.; Waseem, M.; Waqas, M.M.; Bhatti, M.A.; Ahmad, A. Optimizing management options through empirical modeling to improve pearl millet production for semi-arid and arid regions of Punjab, Pakistan. *Sustainability* **2020**, *12*, 7715. [CrossRef]
33. Gerdemann, J.W.; Nicolson, T.H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **1963**, *46*, 235–244. [CrossRef]

34. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; Circular No.939; U.S. Department of Agriculture: Washington DC, USA, 1954.
35. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
36. Tahat, M.M.; Kamaruzaman, S.; Radziah, O.; Kadir, J.; Masdek, H.N. Response of *Lycopersicum esculentum* Mill. to Different arbuscular mycorrhizal fungi species. *Asian J. Plant Sci.* **2008**, *7*, 479–484. [CrossRef]
37. Kazemi, R.; Ronaghi, A.; Yasrebi, J.; Ghasemi-Fasaei, R.; Zarei, M. Effect of shrimp waste–derived biochar and arbuscular mycorrhizal fungus on yield, antioxidant enzymes, and chemical composition of corn under salinity stress. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 758–770. [CrossRef]
38. Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [CrossRef]
39. Biermann, B.; Linderman, R.G. Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles, and extra radical vesicles as inoculum. *New Phytol.* **1983**, *95*, 97–105. [CrossRef]
40. Morton, J.B.; Benny, L. Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*) a new order, Glomales, two new sub-orders Glomineae and Gaigasporineae, and two new families, *Acaulosporaceae* and *Gigasporaceae* with an emendation of *Glomaceae*. *Mycotaxan* **1990**, *37*, 471–491.
41. Pérez, Y.; Schenck, N.C. A unique code for each species of VA mycorrhizal fungi. *Mycologia* **1990**, *82*, 256–260. [CrossRef]
42. Hall, I.R.; Fish, B.J. A key to the *Endogonaceae*. *Trans. Br. Mycol. Soc.* **1979**, *73*, 261–270. [CrossRef]
43. Simpson, E.H. Measurement of diversity. *Nature* **1949**, *163*, 168. [CrossRef]
44. Steel, R.G.D.; Torrie, J.H.; Dicky, D.A. *Principles and Procedures of Statistics—A Biometrical Approach*; McGraw-Hill: Singapore, 1997; pp. 204–227.
45. Yasmeen, T.; Tariq, M.; Iqbal, S.; Arif, M.S.; Riaz, M.; Shahzad, S.M.; Ali, S.; Noman, M.; Li, T. Ameliorative Capability of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) Against Salt Stress in the Plant. In *Plant Abiotic Stress Tolerance*; Springer: Berlin, Germany, 2019.
46. Parihar, P.; Bora, M. Effect of mycorrhiza (*Glomus mosseae*) on morphological and biochemical properties of Ashwagandha (*Withania somnifera*) (L.) Dunal. *J. Appl. Nat. Sci.* **2018**, *10*, 1115–1123. [CrossRef]
47. Sivakumar, N. Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscular mycorrhizal fungi in sugarcane fields. *Ann. Microbiol.* **2013**, *63*, 151–160. [CrossRef]
48. Begum, N.; Qin, C.; Ahanger, M.A.; Raza, S.; Khan, M.I.; Ashraf, M.; Ahmed, N.; Zhang, L. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Front. Plant Sci.* **2019**, *10*, 1068. [CrossRef] [PubMed]
49. Azcon, R.B.; Ferrol, N.; Azcón-Aguilar, C. Ecological and functional roles of mycorrhizas in semiarid ecosystems of Southeast Spain. *J. Arid Environ.* **2011**, *75*, 1292–1301.
50. Panwar, J.; Tarafdar, J.C. Arbuscular mycorrhizal fungal dynamics under *Mitragyna parvifolia* (Roxb.) Korth. in the Thar Desert. *Appl. Soil Ecol.* **2006**, *34*, 200–208. [CrossRef]
51. Mehboob; Vyas, A. Diversity of am fungi in the rhizosphere of *Trigonellafoenum greacum* in western Rajasthan. *Int. J. Plant Anim. Environ. Sci.* **2013**, *3*, 38–43.
52. Hontoria, C.; García-González, I.; Quemada, M.; Roldán, A.; Alguacil, M.M. The cover crop determines the AMF community composition in the soil and roots of maize after a ten-year continuous crop rotation. *Sci. Total Environ.* **2019**, *660*, 913–922. [CrossRef]
53. Cofré, N.; Becerra, A.G.; Marro, N.; Domínguez, L.; Urcelay, C. Soybean growth and foliar phosphorus concentration mediated by arbuscular mycorrhizal fungi from soils under different no-till cropping systems. *Rhizosphere* **2020**, *16*, 100254. [CrossRef]
54. Manoharan, L.; Rosenstock, N.P.; Williams, A.; Hedlund, K. Agricultural management practices influence AMF diversity and community composition with cascading effects on plant productivity. *Appl. Soil Ecol.* **2017**, *115*, 53–59. [CrossRef]
55. Zhu, X.; Yang, W.; Song, F.; Li, X. Diversity and composition of arbuscular mycorrhizal fungal communities in the cropland black soils of China. *Glob. Ecol. Conserv.* **2020**, *22*, e00964. [CrossRef]
56. Shukla, A.; Kumar, A.; Prasad, R.; Kumar, N.; Dhyani, S.K.; Chaturvedi, O.P.; Arunachalam, A. Efficacy of arbuscular mycorrhizal fungi and bacterial inoculants in enhancing yield of *Phaseolus mungo* L. and *Vigna radiata* (L.) R. Wilczek under central Indian conditions. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 1559–1571. [CrossRef]
57. Zhang, W.; Liu, D.; Liu, Y.; Cui, Z.; Chen, X.; Zou, C. Zinc uptake and accumulation in winter wheat relative to changes in root morphology and mycorrhizal colonization following varying phosphorus application on calcareous soil. *Field Crop. Res.* **2016**, *197*, 74–82. [CrossRef]
58. Liang, J.-F.; An, J.; Gao, J.-Q.; Zhang, X.-Y.; Song, M.-H.; Yu, F.-H. Interactive effects of biochar and AMF on plant growth and greenhouse gas emissions from wetland microcosms. *Geoderma* **2019**, *346*, 11–17. [CrossRef]
59. Evelin, H.; Kapoor, R.; Giri, B. Arbuscular mycorrhizal fungi in alleviation of salt stress: A review. *Ann. Bot.* **2009**, *104*, 1263–1280. [CrossRef]
60. Mathur, N.; Singh, J.; Bohra, S.; Vyas, A. Arbuscular mycorrhizal status of medicinal halophytes in saline areas of Indian Thar desert. *Int. J. Soil Sci.* **2007**, *2*, 119–127.
61. Alloush, G.A.; Zeto, S.K.; Clark, R.B. Phosphorus source, organic matter, and arbuscular mycorrhiza effects on growth and mineral acquisition of chickpea grown in acidic soil. *J. Plant Nutr.* **2000**, *23*, 1351–1369. [CrossRef]

62. Zaidi, A.; Khan, M.S.; Amil, M. Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *Eur. J. Agron.* **2003**, *19*, 15–21. [CrossRef]
63. Akhtar, M.S.; Siddiqui, Z.A. Effects of *Glomus fasciculatum* and *Rhizobium* sp. on the growth and root-rot disease complex of chickpea. *Arch. Phytopathol. Plant Protect.* **2007**, *40*, 37–43. [CrossRef]
64. Chaudhry, S.; Luhach, J.; Sharma, V.; Sharma, C. Assessment of diesel degrading potential of fungal isolates from sludge contaminated soil of petroleum refinery, Haryana. *Res. J. Microbiol.* **2012**, *7*, 182–190. [CrossRef]
65. Bohrer, G.; Kagan-Zur, V.; Roth-Bejerano, N.; Ward, D. Effects of environmental variables on the vesicular-arbuscular mycorrhizal abundance in wild populations of *Vangueria infausta*. *J. Veg. Sci.* **2001**, *12*, 279–288. [CrossRef]
66. Muthukumar, T.; Udaiyan, K. Seasonality of vesicular-arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. *Acta Oecol.* **2002**, *23*, 337–347. [CrossRef]
67. Elmostapha, O.; Hanane, D.; Rachid, B.; Lahcen, O. Application of arbuscular mycorrhizal fungi isolates from semi-arid Mediterranean ecosystems as biofertilizers in argan tree development. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 944–955. [CrossRef]