



## Unravelling the Diversity, Root Colonization, and Morphological Features of Arbuscular Mycorrhizal Fungi Associated with Invasive Plant Species of Sirmaur, Himachal Pradesh, India

Kaur M and Singh PK\*

Department of Botany, Eternal University, Baru Sahib, Sirmaur- 173 101, Himachal Pradesh, India

Kaur M, Singh PK 2024 – Unravelling the Diversity, Root Colonization, and Morphological Features of Arbuscular Mycorrhizal Fungi Associated with Invasive Plant Species of Sirmaur, Himachal Pradesh, India. Asian Journal of Mycology 7(2), 1–10, Doi 10.5943/ajom/7/2/1

### Abstract

This study assessed the diversity and colonization patterns of arbuscular mycorrhizal fungi (AMF) associated with selected invasive plant species, i.e., *Parthenium*, *Cannabis*, *Mimosa*, *Lantana*, and *Asclepias* spp. Soil samples were collected from the rhizosphere of five invasive plants in the Baru Sahib area, Sirmaur, Himachal Pradesh, India. The samples were analyzed using various methods, including wet-sieving, decanting, and microscopy. Fifty-six morphologically distinct AMF species belonging to 15 genera were identified. *Glomus* species were the most abundant, followed by *Acaulospora* and *Rhizophagus*. *Parthenium hysterophorus* exhibited the highest root colonization percentage (94.8%), while *Asclepias curassavica* had the lowest (61.6%). *Lantana camara* had the highest number of AMF spores in the soil samples, while *Cannabis sativa* had the lowest spore number. These findings provide valuable insights into the diversity and colonization patterns of AMF associated with invasive plant species, which can contribute to our understanding of the ecological interactions between invasive plants and soil microbial communities.

**Keywords** – Ecological impacts – Mycorrhization – Root Colonization – Symbiotic associations

### Introduction

Invasive plant species pose significant threats to natural ecosystems, agricultural productivity, and biodiversity. One key factor that influences the success of invasive plants is their interaction with beneficial soil microorganisms, including arbuscular mycorrhizal fungi (AMF). AMF forms symbiotic associations with plant roots, providing numerous ecological benefits such as enhanced nutrient uptake, improved drought tolerance, and increased resistance to pathogens (Smith & Read 2008, Singh et al. 2024, Berruti et al. 2016). Understanding the diversity, root colonization patterns, and morphological features of AMF associated with invasive plant species is crucial for comprehending their ecological impacts and developing effective management strategies.

The diversity of AMF is known to vary across different plant species and ecosystems (Vandenkoornhuysen et al. 2010). Numerous studies have explored AMF diversity in native plant communities; however, limited research has focused specifically on AMF diversity associated with invasive plant species (Singh et al. 2011, Thakur et al. 2022, Chen et al. 2023). Investigating the diversity of AMF associated with invasive plants is essential, as it can shed light on the potential factors driving their invasiveness and provide insights into the mechanisms by which they out-compete native plants (Powell et al. 2009). Some notable invasive plant species that have been

associated with AMF include *Asclepias curassavica* L., *Cannabis sativa* L., *Mimosa pudica* L., *Lantana camara* L., and *Parthenium hysterophorus* L. (Kaur 2022). These plants have shown varying degrees of invasiveness and have been found to interact with AMF in different ways (van der Putten et al. 2013). *Asclepias curassavica* L., commonly known as tropical milkweed, has become invasive in some regions and has been observed to have high levels of root colonization by AMF (Liu et al. 2020). *Cannabis sativa* L., a widely cultivated and invasive plant species, has also been found to establish symbiotic associations with AMF (Tian et al. 2018). *Mimosa pudica* L., *Lantana camara* L., and *Parthenium hysterophorus* L. are notorious invasive plant species that have significant ecological impacts and may exhibit distinct patterns of AMF root colonization and morphological features (Klironomos et al. 2000, Zhang et al. 2019).

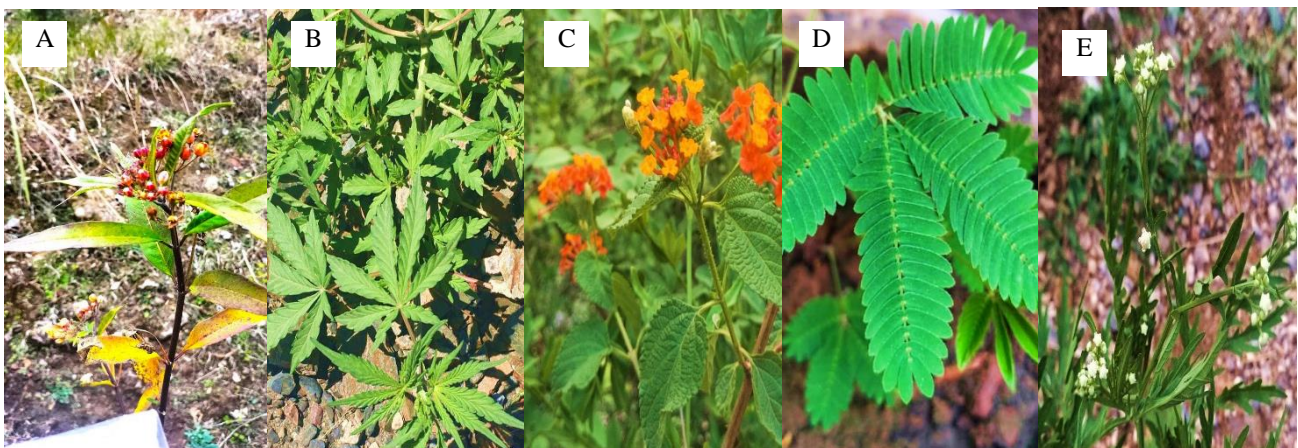
This research paper aims to unravel the diversity, root colonization patterns, and morphological features of AMF associated with invasive plant species, including *A. curassavica*, *C. sativa*, *M. pudica*, *L. camara*, and *P. hysterophorus* in Baru Sahib, Himachal Pradesh, India. By elucidating these aspects, we can deepen our understanding of the complex interactions between invasive plants and AMF and their ecological implications. This knowledge can guide future conservation efforts, invasive species management, and restoration practices aimed at mitigating the impacts of invasive plants.

## Materials & Methods

For the investigation of diversity, root colonization, and morphological study of AMF associated with invasive alien species, the current analysis was conducted at Eternal University, located in Baru Sahib, District Sirmaur, Himachal Pradesh (Table 1, Fig. 1). The study region of Baru Sahib is situated at the latitude of 30.8244 North and a longitude of 77.26855 East.

**Table 1** Selected Invasive Alien Plant species for study with their locations.

S. No.	Selected Plants	Location	
		Latitude	Longitude
1	<i>Asclepias curassavica</i> L.	30.753763°N	77.296538°E
2	<i>Cannabis sativa</i> L.	30.806303°N	77.291414°E
3	<i>Lantana camara</i> L.	30.755216°N	77.296496°E
4	<i>Mimosa pudica</i> L.	30.758432°N	77.296855°E
5	<i>Parthenium hysterophorus</i> L.	30.755543°N	77.296762°E



**Fig. 1** – Selected Invasive Alien Plant species for study. A *Asclepias curassavica*. B *Cannabis sativa*. C *Lantana camara*. D *Mimosa pudica*. E *Parthenium hysterophorus*.

### Collection of soil samples, Extraction, Quantification, and Identification of AMF spores

Soil samples were collected from the rhizosphere of five invasive plant species. After removing the top soil layer, the soil samples were taken by digging out a small amount of soil close to a plant at a depth of 10–15 cm. Plant soil samples were collected in transparent polythene bags and carried aseptically to the research lab. The isolation of AMF spores from the soil samples was performed using wet-sieving and decanting methods (Gerdemann & Nicolson 1963). The quantification of AMF spores was done by the method of Gour & Adholleya (1994). The AMFs were identified using several identification keys (Schenck & Perez 1990) and [www.amf.phylogeny.com](http://www.amf.phylogeny.com).

### Collection of root samples, Staining, and Root Colonization Assessment

The roots were gently excavated with a hoe, tending not to damage the tiny roots. Separated fine feeder roots were gently shaken and delivered to the lab in labelled polythene bags, with the root samples that emerged in an FAA (Formalin-Acetic Acid-Alcohol) for further examination. Clearing methods, which utilize chemical agents to remove cell contents and cell wall pigments, are often used to see interior characteristics in plant tissues (Phillips & Hayman 1970). The grid-line slide technique (Giovannetti & Mosse 1980) was used to assess AMF colonization. Each 1 cm long root segment was chosen at random from stained samples and arranged in groups of ten on microscope slides. The presence and absence of infection were recorded in each of the ten components, with a minimum of 100 root segments employed for this operation. The following calculation was used to compute the proportion of roots colonized:

$$AMF \text{ Root Colonization (\%)} = \frac{\text{Number of root pieces with AMF Structure}}{\text{Number of root pieces examined}} \times 100$$

### Frequency, Density, and Relative Density of AM fungi

The frequency, density, and relative density were calculated by using the following formula:

$$\text{Frequency (\%)} = \frac{\text{Number of hosts having AMF}}{\text{Total number of host examined}} \times 100$$

$$\text{Density} = \frac{\text{Number of individuals having AMF with all fields}}{\text{Total number of fields examined}}$$

$$\text{Relative density} = \frac{\text{Density}}{\text{Total Density}} \times 100$$

### Statistical analysis

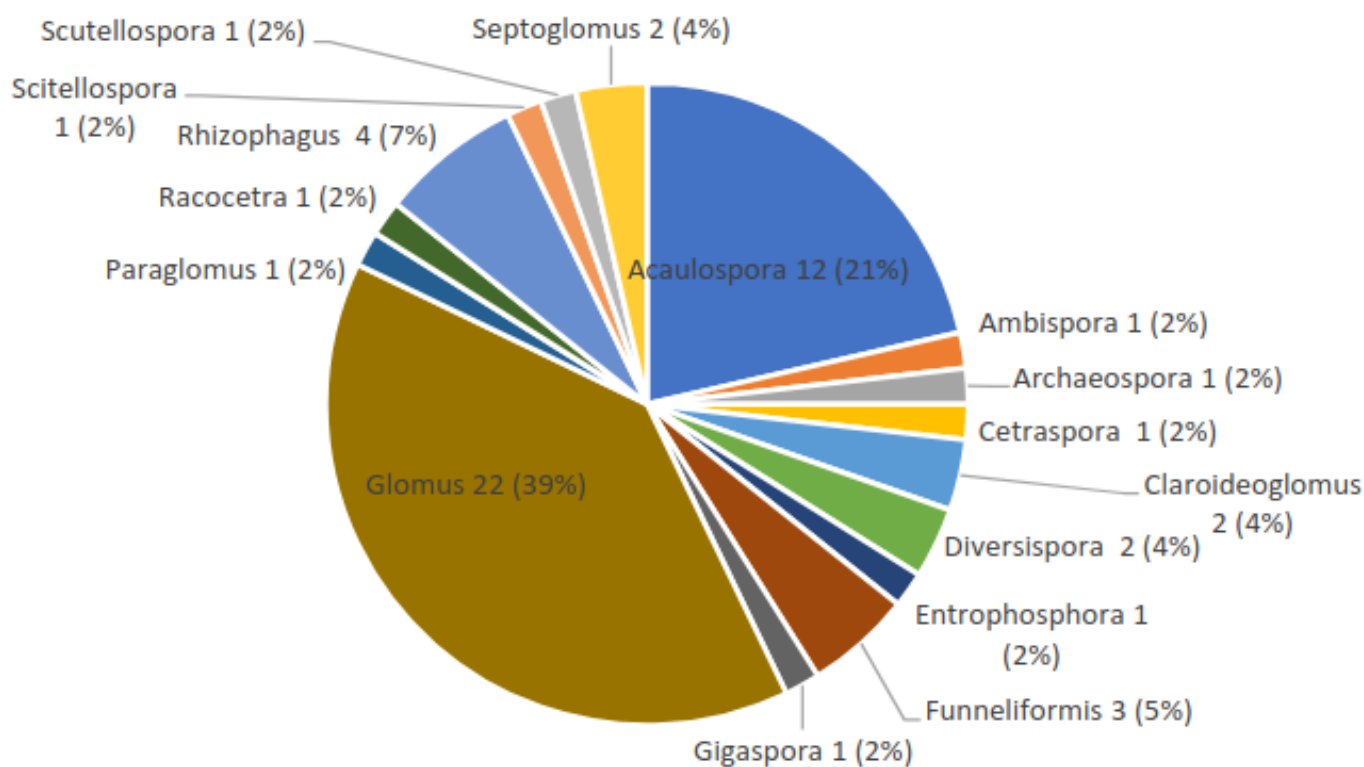
Following Gupta & Kapoor (1997), data were statistically analyzed for means comparison, analysis of variance (ANOVA), least significant difference (LSD), critical difference between two means (C.D.), etc.

### Results

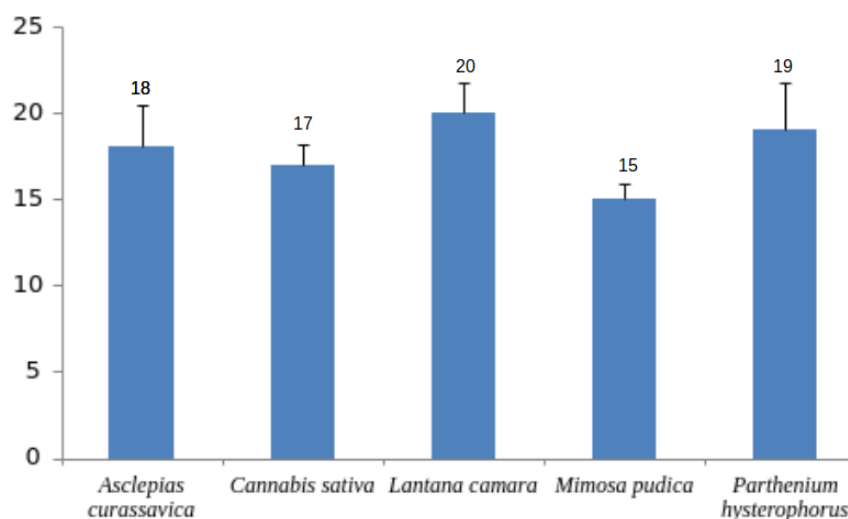
A total of 56 morphologically distinct species of AMF belonging to 15 genera were observed in our study (Table 2, Fig. 2). The genus *Glomus* exhibited the highest number of species, with a total of 22 species. *Acaulospora* was the second most abundant genus, comprising 12 species. On the other hand, nine genera, *Ambispora*, *Archaeospora*, *Cetraspora*, *Entrophosphora*, *Gigaspora*, *Paraglomus*, *Racocetra*, and *Septoglomus*, were represented by only one species each. *Rhizophagus*, *Funneliformis*, *Claroideoglomus*, *Diversispora*, *Scutellospora*, and *Septoglomus* fell in between, with two to four species each (Fig. 3). These findings highlight the rich diversity of AMF species and the varied representation of different genera associated with the invasive alien plant species under investigation. It emphasizes the importance of considering the intricate interactions between AMF and invasive plants in understanding their ecological dynamics and potential impacts on ecosystems.

**Table 2** Arbuscular mycorrhizal fungi species associated with invasive plant species

Invasive Plant Species	AMF Species
<i>Asclepias curassavica</i>	<i>Acaulospora elegans</i> , <i>A. koskei</i> , <i>A. spinosa</i> , <i>A. tuberculata</i> , <i>A. soloidea</i> , <i>Archaeospora trappei</i> , <i>Cetraspora pellucida</i> , <i>Claroideoglossum luteum</i> , <i>Diversispora spurca</i> , <i>Gigaspora albida</i> , <i>Glomus aggregatus</i> , <i>G. albidum</i> , <i>G. fistulosum</i> , <i>G. macrocarpum</i> , <i>G. pansiholos</i> , <i>G. pustulotam</i> , <i>Rhizophagus fasciculatus</i> , <i>R. invarmaium</i>
<i>Cannabis sativa</i>	<i>A. gerdemini</i> , <i>A. mellea</i> , <i>A. spinosa</i> , <i>Archaeospora trappii</i> , <i>Funneliformis mosseae</i> , <i>G. caledonius</i> , <i>G. castrictum</i> , <i>G. clasum</i> , <i>G. climorphicum</i> , <i>G. tunieatum</i> , <i>G. globiferum</i> , <i>G. hoi</i> , <i>G. monosporum</i> , <i>G. pansihalos</i> , <i>Racocetra gregaria</i> , <i>Scutellospora calospora</i> , <i>Septoglossum deserticola</i>
<i>Lantana camara</i>	<i>A. lacunose</i> , <i>A. mellea</i> , <i>A. myriocarpa</i> , <i>A. scrobiculata</i> , <i>Claroideoglossum lamellosum</i> , <i>Diversispora globifera</i> , <i>Entrophosphora colombiana</i> , <i>Funneliformis coronatum</i> , <i>Funneliformis geosporum</i> , <i>Funneliformis mosseae</i> , <i>G. aggregatus</i> , <i>G. ambisporum</i> , <i>G. botryoides</i> , <i>G. clasum</i> , <i>G. climorphicum</i> , <i>G. hoi</i> , <i>G. mosseae</i> , <i>G. pallidum</i> , <i>Scutellospora scutate</i> , <i>Septoglossum deserticola</i>
<i>Mimosa pudica</i>	<i>A. berticulata</i> , <i>A. gerdemini</i> , <i>A. mellea</i> , <i>A. soloidea</i> , <i>A. spinosa</i> , <i>Ambispora leptoticha</i> , <i>Entrophosphora colombianas</i> , <i>Funneliformis mossese</i> , <i>G. austral</i> , <i>G. botryoides</i> , <i>G. clarum</i> , <i>G. climorphicum</i> , <i>G. hoi</i> , <i>G. monosporum</i> , <i>Scutellospora calospora</i>
<i>Parthenium hysterophorus</i>	<i>A. foveate</i> , <i>A. mellea</i> , <i>A. scrobiculata</i> , <i>A. leptoticha</i> , <i>Diversispora globifera</i> , <i>Funneliformis coronatus</i> , <i>Funneliformis mossese</i> , <i>Gigaspora albida</i> , <i>G. calocarpa</i> , <i>G. clarum</i> , <i>G. clavisporem</i> , <i>G. fistulosum</i> , <i>G. hoi</i> , <i>G. pansihalos</i> , <i>G. truffemii</i> , <i>Paraglossum oculatum</i> , <i>Rhizophagus aggrigatus</i> , <i>Rhizophagus intraradices</i> , <i>Septoglossum constrictum</i>



**Fig. 2** – Number of species in different genera of arbuscular mycorrhizal fungi



**Fig. 3** – Number of arbuscular mycorrhizal fungi associated with invasive plant species. The number of AMF species with each plant is shown at the top of the error bar.

Table 3 provides the density, frequency, and Importance Value Index (IVI) of the selected AMF species. Among the 56 AMF species observed, none of them exhibited a frequency of 100%. However, four AMF species displayed the highest frequency of 80%, indicating their presence in four different plant species. These four species are *A. mellea*, *F. mosseae*, *G. clarum*, and *G. hoi*. These species also demonstrated high density (0.8), abundance (7.14), and IVI value (43.57), highlighting their significant presence and importance in the studied ecosystem. On the other hand, several AMF species exhibited the lowest frequency of 20%, as well as low density (0.2), abundance (1.79), and IVI (10.89), indicating their limited occurrence in only one plant species. These variations in frequency, density, abundance, and IVI values reflect the diverse distribution and ecological significance of different AMF species within the rhizosphere of the studied plant species.

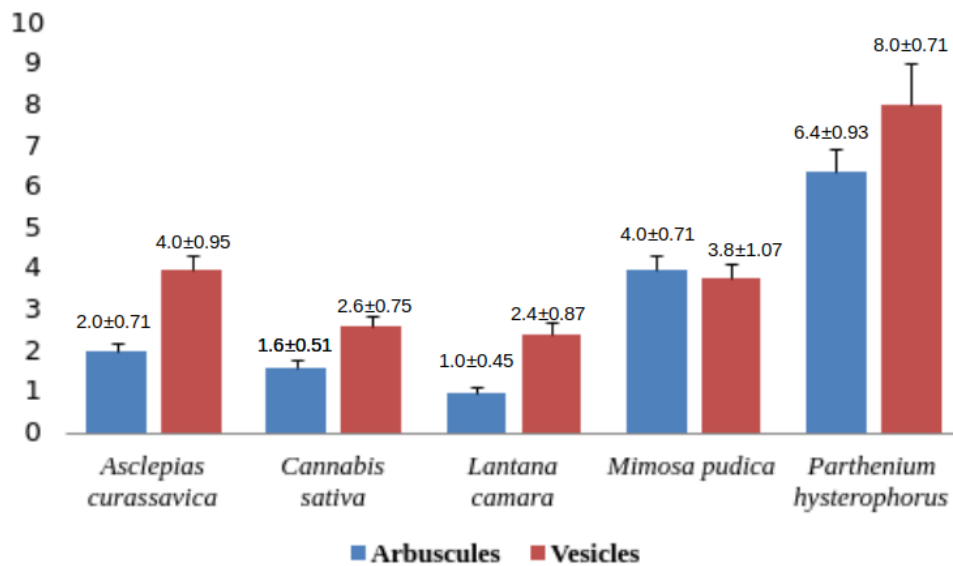
**Table 3** Density, frequency, relative abundance and important value index of AMF species.

No.	AMF species	Invasive Alien species					No. of host	Density	Relative density	Frequency (%)	Relative abundance	Importance value index (IVI)
		LC	CS	AC	MP	PH						
1	<i>Acaulospora berticulata</i>				1		1	0.2	1.12	20	1.79	10.89
2	<i>A. elegans</i>			1			1	0.2	1.12	20	1.79	10.89
3	<i>A. foveate</i>					1	1	0.2	1.12	20	1.79	10.89
4	<i>A. gerdeminii</i>		1				2	0.4	2.25	40	3.57	21.79
5	<i>A. koskei</i>			1			1	0.2	1.12	20	1.79	10.89
6	<i>A. lacunose</i>	1					1	0.2	1.12	20	1.79	10.89
7	<i>A. mellea</i>	1	1		1	1	4	0.8	4.49	80	7.14	43.57
8	<i>A. myriocarpa</i>	1					1	0.2	1.12	20	1.79	10.89
9	<i>A. scorbiculata</i>	1				1	2	0.4	2.25	40	3.57	21.79
10	<i>A. soloidea</i>			1	1		2	0.4	2.25	40	3.57	21.79
11	<i>A. spinosa</i>		1	1	1		3	0.6	3.37	60	5.36	32.68
12	<i>A. tuberculata</i>			1			1	0.2	1.12	20	1.79	10.89
13	<i>Ambispora leptoticha</i>				1	1	2	0.4	2.25	40	3.57	21.79
14	<i>Archaeospora trappei</i>		1	1			2	0.4	2.25	40	3.57	21.79
15	<i>Cetraspora pellucida</i>			1			1	0.2	1.12	20	1.79	10.89

**Table 3** Continued.

S. No.	AMF species	Invasive Alien species			No. of host	Density	Relative density	Frequency (%)	Relative abundance	Importance value index (IVI)
16	<i>Claroideoglossum lamellosum</i>	1			1	0.2	1.12	20	1.79	10.89
17	<i>C. luteum</i>		1		1	0.2	1.12	20	1.79	10.89
18	<i>Diversispora globifera</i>	1		1	2	0.4	2.25	40	3.57	21.79
19	<i>D. spurca</i>		1		1	0.2	1.12	20	1.79	10.89
20	<i>Entrophosphora colombiana</i>	1		1	2	0.4	2.25	40	3.57	21.79
21	<i>Funneliformis coronatum</i>	1		1	2	0.4	2.25	40	3.57	21.79
22	<i>F. geosporum</i>	1			1	0.2	1.12	20	1.79	10.89
23	<i>F. mosseae</i>	1	1	1	4	0.8	4.49	80	7.14	43.57
24	<i>Gigaspora albida</i>		1	1	2	0.4	2.25	40	3.57	21.79
25	<i>Glomus aggregatus</i>	1	1		2	0.4	2.25	40	3.57	21.79
26	<i>G. albidum</i>		1		1	0.2	1.12	20	1.79	10.89
27	<i>G. ambisporum</i>	1			1	0.2	1.12	20	1.79	10.89
28	<i>G. austral</i>			1	1	0.2	1.12	20	1.79	10.89
29	<i>G. botryoides</i>	1		1	2	0.4	2.25	40	3.57	21.79
30	<i>G. caledonius</i>		1		1	0.2	1.12	20	1.79	10.89
31	<i>G. calocarpa</i>			1	1	0.2	1.12	20	1.79	10.89
32	<i>G. castrictum</i>		1		1	0.2	1.12	20	1.79	10.89
33	<i>G. clasum</i>	1	1	1	4	0.8	4.49	80	7.14	43.57
34	<i>G. clavisorum</i>			1	1	0.2	1.12	20	1.79	10.89
35	<i>G. climorphicum</i>	1	1	1	3	0.6	3.37	60	5.36	32.68
36	<i>G. etunieatum</i>		1		1	0.2	1.12	20	1.79	10.89
37	<i>G. fistulosum</i>		1	1	2	0.4	2.25	40	3.57	21.79
38	<i>G. globiferum</i>		1		1	0.2	1.12	20	1.79	10.89
39	<i>G. hoi</i>	1	1	1	4	0.8	4.49	80	7.14	43.57
40	<i>G. monosporum</i>		1		1	0.2	1.12	20	1.79	10.89
41	<i>G. macrocarpum</i>		1	1	2	0.4	2.25	40	3.57	21.79
42	<i>G. mosseae</i>	1			1	0.2	1.12	20	1.79	10.89
43	<i>G. pallidum</i>	1			1	0.2	1.12	20	1.79	10.89
44	<i>G. pansihalos</i>		1	1	3	0.6	3.37	60	5.36	32.68
45	<i>G. pustulotam</i>		1		1	0.2	1.12	20	1.79	10.89
46	<i>G. trufemii</i>			1	1	0.2	1.12	20	1.79	10.89
47	<i>Paraglomus occulatum</i>			1	1	0.2	1.12	20	1.79	10.89
48	<i>Racocetra gregaria</i>		1		1	0.2	1.12	20	1.79	10.89
49	<i>Rhizophagus aggrigatus</i>			1	1	0.2	1.12	20	1.79	10.89
50	<i>R. fasciculatus</i>		1		1	0.2	1.12	20	1.79	10.89
51	<i>R. intraradices</i>			1	1	0.2	1.12	20	1.79	10.89
52	<i>R. invarmaium</i>		1		1	0.2	1.12	20	1.79	10.89
53	<i>Scitellospora calospora</i>		1	1	2	0.4	2.25	40	3.57	21.79
54	<i>Scutellospora scutate</i>	1			1	0.2	1.12	20	1.79	10.89
55	<i>Septoglossum constrictum</i>			1	1	0.2	1.12	20	1.79	10.89
56	<i>S. deserticola</i>	1	1		2	0.4	2.25	40	3.57	21.79

*Parthenium hysterophorus* exhibits the highest number of spores and vesicles among the studied plant species, followed by *M. pudica*. In contrast, *L. camara* shows the lowest number of vesicles and arbuscules. On average, *A. curassavica* displays two arbuscules and four vesicles, while *C. sativa* has an average of 1.6 arbuscules and 2.6 vesicles (Fig. 4). These observations indicate variations in the abundance of spores, vesicles, and arbuscules across the different plant species.



**Fig. 4** – Number of arbuscules and vesicles of AMF associated with invasive plant species. The number of arbuscules and vesicles is shown at the top of the error bar.

Among the selected plants, *P. hysterophorus* demonstrated the highest root colonization percentage of 94.8%, indicating a strong association with AMF species. *Cannabis sativa* exhibited a lower but significant root colonization percentage of 75.4%. *Asclepias curassavica* displayed the least amount of AMF root colonization, with a percentage of 61.6%. *Lantana camara* and *M. pudica* fell between these values, showing root colonization percentages of 71.4% and 73%, respectively (Fig. 5). These findings highlight the varying degrees of AMF colonization among the studied plant species.

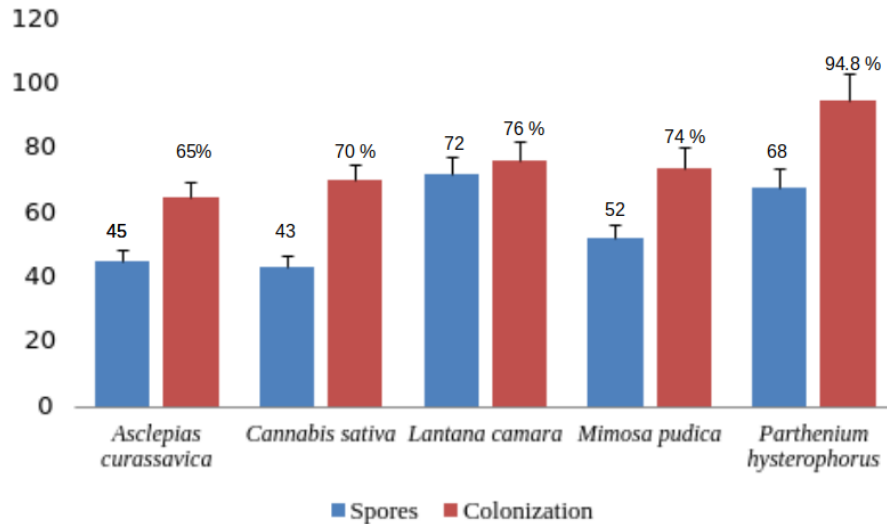
In the current study, a soil sample of 50 g was taken for analysis. *Lantana camara* exhibited significantly high spore numbers, with a total of 72 AMF spores per 50 g of soil, followed by *P. hysterophorus* with 68 AMF spores and *M. pudica* with 52 AMF spores. Moreover, *C. sativa* spore numbers are significantly lower ( $p=0.05$ ) among the selected plants, with 43 AMF spores, followed by *A. curassavica* with 45 AMF spores (Fig. 5). These findings highlight the variation in AMF species abundance within the studied plant species.

## Discussions

The present study aimed to investigate the diversity and colonization patterns of AMF associated with selected invasive plant species. The results reveal significant differences in the species composition and abundance of AMF among the studied plant species. These findings have important implications for understanding the ecological interactions between invasive plants and AMF communities.

*Lantana camara* exhibited the highest number of AMF species, with 72 spores recorded in the 50 g soil sample. This finding suggests that *L. camara* has diverse AMF communities, which may contribute to its successful invasion in the study area (Smith et al. 2011). The presence of many AMF species in the rhizosphere of *L. camara* could enhance nutrient uptake and promote plant growth, giving it a competitive advantage over native species (Smith et al. 2011, Lin et al. 2015). *Parthenium hysterophorus*, another invasive plant species, also exhibited a high number of AMF species, with a

total of 68 spores recorded. This result is consistent with previous studies that have shown the importance of AMF in the successful invasion of *P. hysterophorus* (Qi et al. 2022). The high colonization rate of AMF in *P. hysterophorus*, as evidenced by the presence of arbuscules and vesicles, suggests a mutualistic relationship between this plant species and the AMF community (Zhang et al. 2017).



**Fig. 5** – Number of AMF spores (50 g soil) and root colonization (%) with invasive plant species. The numbers of spores and percent root colonizations are shown at the top of the error bars.

In contrast, *M. pudica* had a lower number of AMF species compared to *L. camara* and *P. hysterophorus*, with a total of 52 spores observed. This finding indicates a potential difference in the extent of AMF association and its role in facilitating the invasiveness of *M. pudica* compared to the other two invasive species (Li et al. 2006). Further research is needed to explore the functional significance of the identified AMF species and their contribution to the invasiveness of *M. pudica*. In our study, we observed a notably higher AMF colonization rate compared to many previous studies, which reported colonization rates ranging from 21% to 73% (Li et al. 2006, Balzergue et al. 2011, Hack et al. 2019). This disparity could be attributed to the elimination of interference from other phosphorus-solubilizing bacteria in our axenic systems (Spagnoletti et al. 2017). It is possible that plants allocate additional resources to sustain and maintain a mutualistic relationship with AMF.

*Asclepias curassavica* and *C. sativa* showed relatively lower numbers of AMF species, with 45 and 43 spores recorded, respectively. These findings suggest that these two invasive plant species may have a less prominent reliance on AMF for their growth and establishment compared to *L. camara* and *P. hysterophorus*. However, it is important to note that the presence of even a moderate number of AMF species in these plants indicates some level of mycorrhizal association, which can still have ecological implications (Zhang et al. 2016). Previous studies have examined biomass gain in realistic scenarios where invasive forbs invade native grasslands and found contrasting effects of AMF. Some studies have reported that AMF enhanced the growth of invasive forb species (Zabinski et al. 2002, Zhang et al. 2017, Thakur et al. 2022), while others have found that the absence of AMF promoted the growth of invasive plants (Waller et al. 2016).

The results revealed significant differences in the species composition and abundance of AMF among the studied plant species. These findings indicate that invasive plant species differ in their associations with AMF communities, which may contribute to their successful invasion in the study area. The presence of a diverse AMF community in invasive plants can confer advantages such as enhanced nutrient uptake and competitiveness. However, further research is needed to understand the functional implications of specific AMF species and their interactions with invasive plants. These



findings have important implications for the management and restoration of invaded ecosystems by providing insights into the ecological interactions between invasive plants and AMF communities.

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