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Diversity and distribution of arbuscular mycorrhizal fungi associated with vegetable crops in Haryana, India Anju Tanwar a,*, Ashok Aggarwal ^b, Ishan Saini ^c , Tarsem Kumar ^d, Mukesh Kumar ^e, Sergio T. Pichardo ^f

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Abstract

Article Info The optimal growth and development of many vegetable crops hinge significantly upon their reliance on Arbuscular Mycorrhizal Fungi (AMF). Understanding the AMF status of vegetable crops can assist researchers in selecting suitable strains for future experiments. Therefore, a field work was carried out to determine the species diversity and composition of AMF with fifty vegetable crops from seventeen different districts of Haryana. AMF spores were isolated and identified to evaluate AMF density, diversity, and host preference in terms of AMF species richness, abundance and frequency of occurrence. Soil conditions, land use type and its physico–chemical properties played a crucial role in regulating the uneven distribution and composition of AMF. Mycotrophic structures such as linear infection (Arum–type) to coils (Paris– type) arbuscules and vesicles were seen. Interestingly, no correlation was found between spore number and root colonization. Maximum AMF spore density, spore richness and abundance were witnessed in *Zea mays* and *Trigonella foenum–graecum*. Five plants exhibited 100% AMF colonized roots, 15 plants showed above 75% and 12 plants above 50% colonization. Soil pH 6.10 to 7.40 supported the maximal abundance

and frequency of occurrence of *Glomus* and *Acaulospora* with 53 species and 18 ***** Corresponding author species followed by *Acaulospora* (18), *Sclerocystis* (10), *Gigaspora* (5), *Entrophospora* (4) and *Sclerocystis* (4). *G. mosseae* was the most preferred species among vegetable crops. Members of non–mycorrhizal families lack root colonization except for *Brassica campestris*, *B. oleracea* var. *botrytis* and *B. Rapa* where 2–11% root colonization was detected. Noticing the abundant AMF diversity of vegetable crops , this investigation expands the scope of detection, selection and inoculation of vegetable crops with suitable AMF species for improving their quality and quantity.

> **Keywords:** Abundance, Frequency of occurrence, Mycorrhizal distribution, Soil properties, Species richness.

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Introduction

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Exploring the dynamic nexus of plant-root interactions and soil microbiota in the rhizosphere unveils a realm of hidden complexities shaping plant health, nutrient cycling, and ecosystem resilience. Amidst various microbes influencing plants growth and production, the most pervasive and elemental type of relationship is that formed by the fungal endophyte Arbuscular Mycorrhizal Fungi (AMF). Ecological functions of AMF hyphae, have great impacts on global sustainability (Wang et al., 2022). It plays crucial roles in ecosystem

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functioning, inclusive of absorption and transport of mineral nutrients (especially P), maintaining soil fertility, amendment of the physical soil ecosystem, modification of plant association with other biota, maintenance of biodiversity and ecosystem stability together with ecological system protection, restoration, and reconstruction (Powell and Rillig, 2018). AMF have significant prominence for agricultural sustainability owing to their multifaceted contribution in assisting plant growth and productivity. This association improves soil properties, nutrient cycling, plants hormonal regulation and defense mechanisms, AMF are widespread in distribution in both natural and agricultural soil as well with almost 230 AMF species of the phylum Glomeromycota are reported till now (Higo et al., 2013). Perhaps that is why they show great diversity within species, genetic and functional as well. Although, the AMF diversity fluctuates from one type of soil to another type of soil and extremely influenced by the type of host plant, physico–chemical and biota of the experimental soil and other growing mediums also (Tanwar et al., 2021). Researchers are of diverse opinion regarding AMF host specificity which ranges from high host specificity (Yang et al., 2012), certain level of host specificity (Bi et al., 2020) to non–host specificity (Santos–González et al., 2006). Moreover, their population is remarkably influenced by both biotic and abiotic communities as well (Wahab et al., 2023). Factors like available nutrient (Johnson et al., 2015), climatic (Li et al., 2010), forage cover (Pellegrino et al., 2020), elevation (Haug et al., 2019), land–use (Melo et al., 2020) can determine the AMF–plant interactions. According to Ma et al. (2023) latitude and soil available phosphorus are the most important predictors of AMF diversity. Given the growing need for sustainable agricultural practices, understanding the role of AMF in nutrient uptake and soil health is crucial for improving vegetable crop productivity. It is also important to know about the species diversity of the AMF in the plant root to get better insight of the mycorrhizal functioning besides adopting their management and preservation (Alguacil et al., 2009). The evaluation of AMF biodiversity of ecological site is relying on evaluating AMF root colonization and AMF spore number. Beside this, few other factors that contribute to the functioning of agricultural performance is AMF biomass which include spores, extraradical and intraradical hyphae and colonized pieces of roots (Higo et al., 2013). AMF status also relies on the time of sampling, soil depth and growth stage of the test plant which increases from initial vegetative state of the plant to completely grown stage.

In nature, most of the vegetable crops are known to be associated with AMF which directly or indirectly influence their growth and production (Tanwar and Aggarwal, 2014). During the last few decades, different aspects of AMF on vegetable crops have been studied extensively in different geographical and agricultural conditions. Work has also been done on studies related to biodiversity of AMF associated with vegetable crops (Castillo et al., 2016). There are several studies on the AMF status in variety of plants from Indian soil also. However, most of the studies are concentrated either on medicinal plants or fruit crops (Khastini et al., 2020) and our present uderstanding on AMF status and morphology in olericultural crop is limited. Therefore, before applying any commercially available inocula via knowing the AMF status and sustenance of crop plant on particular AMF strain is compulsory. In India some previous studies have mainly focused on AMF diversity in cereal crops, whereas this study specifically targets vegetable crops in the region of Haryana, which has unique soil conditions. Furthermore, the research regarding biodiversity of AMF in vegetable crops mainly comes from southern part of India (Kumar and Garampalli, 2013) and literature perusal did not show any authentic study related to AMF status of vegetable crops of Haryana region. Keeping in view the positive impact of AMF on vegetable crops, it became significantly important to emphasize work on monitoring the mycorrhizal status in vegetable crops. Therefore, this study aims to assess the diversity and colonization rate of AMF associated with different vegetable crops grown under specific agro-ecological conditions in Haryana, India.

Material and Methods

Study Site, Sample Collection and Soil Analysis

Seventeen districts of Haryana (Panchkula, Ambala, Kurukshetra, Yamunanagar, Karnal, Kaithal, Panipat, Sonipat, Jind, Rohtak, Hisar, Bhiwani, Rewari, Faridabad, Gurgaon, Sirsa and Fatehabad) were surveyed for the collection of soil samples and plant roots. Thin roots and soil were randomly collected from the rhizosphere of 50 vegetable crops from various districts of Haryana. For the isolation of AMF spores, the soil samples from five randomly selected plants were collected from the depth of 0–30 cm. All the samples were mixed together to make one composite sample. Collected soil was air dried, grounded and sieved through 2mm sieve and kept at 4–10°C for further analysis. Soil Physico–chemical propertieslike pH, electrical conductivity (EC), organic carbon (OC), available phosphorus (P), potassium (K) and Sulphur (S) were analyzed from Directorate of Agriculture, Krishi Bhawan, Sector–21, Panchkula, Haryana, India (Table 1).

S. no.	Districts	pH	EC $(dS m^{-1})$	OC(%)	P (kg m ⁻²)	K (kg m $^{-2}$)	S(ppm)
1.	Panchkula	6.1	0.32	0.24	3.70	85.0	18.7
2.	Ambala	6.5	0.12	0.25	5.20	88.0	21.2
3.	Karnal	7.0	0.66	0.43	7.50	112.0	21.7
4.	Kurukshetra	6.8	0.25	0.40	7.30	88.0	14.8
5.	Yamunanagar	6.7	0.41	0.16	4.20	78.0	15.7
6.	Kaithal	7.0	0.44	0.35	7.20	77.5	20.2
7.	Panipat	7.0	0.42	0.24	10.30	98.0	28.2
8.	Sonipat	7.1	0.43	0.25	4.40	90.0	27.6
9.	Jind	7.4	0.40	0.12	13.00	78.0	14.9
10.	Rohtak	6.8	0.44	0.48	8.10	125.0	15.7
11.	Hisar	7.0	0.40	0.42	6.80	115.0	15.7
12.	Bhiwani	7.4	0.41	0.16	13.20	78.0	15.7
13.	Rewari	7.4	0.35	0.44	11.80	85.0	34.4
14.	Faridabad	7.1	0.47	0.31	7.30	75.0	24.2
15.	Gurgaon	6.8	0.36	0.18	3.20	72.0	15.7
16.	Sirsa	7.2	0.47	0.45	12.30	75.0	22.4
17.	Fatehabad	7.2	0.30	0.48	13.80	135.0	17.1

Table 1. Physical and chemical characteristics of soil collected from different districts

*Electrical conductivity (EC), organic carbon (OC), available phosphorus (P), potassium (K) and Sulphur (S)

Root Colonization

The fine host roots repeatedly washed with water and then cut to make 1cm long segments. These roots were then processed as per rapid clearing and staining technique by Phillips and Hayman's (1970). Plant root colonization assessment was done by Giovannetti and Mosse's (1980) root slide technique. Individual root fragment was conscientiously examined through its entire length to record mycotrophic structures like intra– radical, extra–radical mycelium, hyphal coils, arbuscules and vesicles, first at 100× then at 400×. The colonized roots were photographed with Nikon Coolpix S4000 camera . The percent root colonization was calculated using the following formula:

> 100 number of root segments studied Percentage AM root colonizati on $=\frac{\text{number of root segments colonized}}{1-\frac{1}{2}} \times$

Isolation and Quantification of AMF Spores

Rhizosphere soil samples were enumerated to detect the presence of AMF spores. Gerdemann and Nicolson's (1963) wet sieving and decanting technique was followed for the isolation of AMF spores. Adoleya and Gaur's (1994) grid line intersect method was followed to quantify AMF spores. Total number of intact spores was counted to calculate spore density and later mounted in polyvinyl lactic acid for identification.

AMF Spore Identification

The spores were identified using the High power research microscope (Suswox Optic, Sudheer Scientific Works–133001, India). The taxonomic identification of AMF spores to species level was done referring the manuals of Schenck and Pérez (1990), Mukerji (1996), Morton and Redecker (2001). Identification was also authenticated from the description in reference cultures in International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi [\(http://invam.cag.wvu.edu\)](http://invam.cag.wvu.edu/) and AMF phylogeny (www.amf–phylogeny.com).

AMF Species Richness, Abundance, and Frequency of Occurrence

Number and type of AMF species was used to calculate AMF species richness, species abundance (A) and frequency of occurrence (FO). Species richness (SR) equals total AMF species number in 50 g soil while species abundance (A) equals number of soil samples having particular species.

FO (%) = number of soil samples possessing spores of particular species
total number of samples analyzed
$$
\times 100
$$

Results

Chemical Analysis of Soil

As per Table 2, the soil pH ranged from 6.10 to 7.40 with minimum with lowest in Panchkula and maximum in Bhiwani, Rewari and Jind. Maximum EC was found in the soil of Karnal (0.66) and minimum in Ambala (0.12) while maximum OC in Rohtak and Fatehabad (0.48) and least in Jind (0.12). Maximum P level was detected in Fatehabad (13.8) and Bhiwani (13.2), while Gurgaon (3.2) had deficient in P. Soil K and S content was also analyzed and none of the soil showed either excess or low level of these nutrients (Table 2).

Each value is a mean of five replicates, ±: standard deviation, A: Arbuscule, M: Mycelium, V: Vesicle, +: present, - : absent

AMF spore density

AMF spore propagule density varied greatly among plants (Table 3). Highest mean spore density (514.2±17.9) was found with *Z. mays* followed by *A. cepa*, *T. foenum–graecum* and *Capsicum annuum* (green and red). While less than 100 spores were seen in the members of Amaranthaceae, Brassicaceae and Chenopodiaceae. Sporocarp of several *Glomus* and *Sclerocystis* species and resting spore i.e., chlamydospores were also seen. AMF spore propagules were strikingly low in the soil of Jind, Bhiwani and Rewari. No direct relationship was

observed between soil nutrient status and mycorrhization except with soil pH and P level. Slightly acidic soil of Panchkula and Gurgaon exhibited higher spore density as compared to samples from alkaline soils of Bhiwani and Jind (Table 3).

Table 3. (Continue)

Occurrence of AMF Morphological Types

The microscopic analysis of the plant root pieces showed the presence of AMF intercellular hyphae, arbuscules, vesicles and hyphal coils (Figure 1). The presence of linear or parallel mycelium characteristic of Arum–type was seen in majority of the vegetable crops. In contrast, some plants showed the presence of Paris– type, which was characterized by the presence of hyphal coils. Prominent hyphal coils were detected in *T. foenum–graecum* and *S. tuberosum* (Figure 1 G, H). Moreover, several other shape of mycelium (H, Y, T, X, lobed, twisted & beaded) was also encountered (Figure 1 K-P). Root penetration through formation of appresorium was clearly detected in the root of *P. sativum* and *C. longa* (Figure 1B) while little bit of extraradical mycelium in the form of parallel running hyphae was detected on the root tips of Brassicaceae (Figure 1C). The details of AMF colonization pattern are furnished in Table 2. Vesicles were detected in majority of the plants while arbuscules were rarely found (Figure 1 E,F). Vesicle shape also showed tremendous variation ranging from round (*A. cepa*), oval (*A. sativum*), beaked (*C. longa*), pear (*Zea mays*), rectangular (*L. esculentum*), elliptical (*C. indica*), triangular (*V. unguiculata*) to irregular (*V. faba*, *T. dioica*) either singly, in pairs and in groups (*C. arietinum* and *T. foenum–graecum*). Likewise, globose vesicles with funnel shaped hyphal attachment were mainly formed in *C. arietinum*.

Extend of AMF Colonization

Maximum colonization was recorded in plants collected from Panchkula, Ambala and Kurukshetra district (Table 2). Based on percentage colonization, plants were classified into those having highest (100%), high $(75-99.9\%)$, moderate $(50-75\%)$, low $(25-50\%)$ and least $(1-25\%)$ colonization. Highest colonization was observed in *A. cepa*, *A. sativum*, *C. arietinum*, *T. foenum–graecum* and *Z. mays* while least was detected in *B. rapa* (2.22±3.04). Seven members of non-mycorrhizal family Brassicaceae showed low colonization (Table 3).

AMF Species Richness, Spore Abundance and Frequency of Occurrence

Data recorded from Table 4 showed maximum AMF species in *T. foenum–graecum* and *Z. mays* (21 each) followed by *A. cepa* (19), while only 3 species were detected in *B. vulgaris* and *B. rapa*. Altogether 94 species representing different genera of AMF i.e., *Acaulospora*, *Entrophospora*, *Glomus*, *Gigaspora*, *Sclerocystis* and *Scutellopsora* were detected. Two genera viz. *Glomus* and *Acaulospora* were dominantly present. Fifty-three species of *Glomus*, 18 of *Acaulospora*, 10 of *Gigaspora*, 5 of *Scutellospora*, 4 each of *Entrophospora* and *Sclerocystis* were detected*.* The most frequent species among vegetable crops was putatively assigned to *Glomus mosseae* (now called as *Funneliformis mosseae*) occurring in 46 studied samples with 92% frequency of occurrence (Table 4).

Table 4. Species abundance and frequency of occurrence of isolated AM fungal species

Figure 1. A: Shows the presence of extensive extramatrical mycelium, B: Appresorium formation and intrusion of AMF hyphae into host roots, C: Colonization of root tip, D: Extensive intramatrical mycelium, E-F: Arbuscules, G-H: Paris-type, I: Germinating AMF spore, J: Extra-radical vesicles, K-P: Different types of intra-radical vesicles, K: Rectangular, L: Small, round & scattered, M: Beaked, N: Big, round & paired, O: Pear, P: Globose with funnel shaped hyphal attachment.

Discussion

Considering the high production cost of olericultural crops, the adoption of AMF inoculation practice can substitute the high input production (Tanwar and Aggarwal, 2014). But before that, knowing the AMF status of crop is important for the selection of efficient strain. All the plant species studied in this investigation showed the presence of AMF association indicating that the AMF are widely distributed in studied site. Yet lot of variation was detected among members of same family, same genera and same locality. This confirms host preference as the prime factor determining AMF symbiosis in soil. Perhaps, abundance of AMF species behaves as indicator species for particular habitats and land sites which might provide certain ecosystem services at their habitats (Oehl et al., 2017). Highest mean spore density was found with *Z. mays* and *A. cepa*. Same results have been observed by Sinegani and Sharifi (2007) and Tran et al. (2019) with maximum AMF spore abundance in *A. cepa* and *A. ampeloprasum* var. *porrum* respectively. Likewise, abundant spore population in the rhizosphere soil of *Capsicum annuun* and *Vigna unguiculata* has been documented by Ríos–Ruiz et al. (2019). Distribution of AMF is a contemporary ecological process. Even some of the plant of same species that differ in age harboured distinctive AMF populations (Husband et al., 2002). Not only the AMF spore density but the AMF spore richness and abundance was also found highest in *Zea mays* and *T. foenum–graecum* followed by *A. cepa* and *L. esculentum* while some plants inhabits quite low AMF spores like members of Cucurbitaceae. Several factors contribute to low AMF population in the soil, including presence of different host plant, application of excess fertilizer and time of sampling (Cassazza et al., 2017).

AMF are considered as essential constituent of soil forming symbiosis with plants roots and positively influence ecosystems functioning (Diagne et al., 2020). Among all the studied plants, 5 plants exhibited 100% root colonization, above 75% and above 50% colonized roots were detected in 15 and 12 plants respectively. The presence of high mycorrhization in vegetable crops is mainly due to the high dependency of these crops on AMF as the mycorrhizal status of crops depends upon the physiological status of the host as well as host genotype (Dickie et al., 2013). Koul et al. (2012) observed 70%, 62% and 75% colonization and 17, 12 and 47 AMF propagules in *A. cepa*, *A. sativum* and *T. foenum–graecum* respectively. The discrepancy in the root

colonization could be accredited to the exudation of some specific metabolites from the plant roots that attract the AMF resulting in disparate colonization pattern amidst different plants (Wen et al., 2019).

It was inferred that the low level of spore population was not related to reduce colonization of roots and likewise plants which do not form AMF colonization inhabits acceptable spore number while few plants harbored prominent spore number which is equivalent to the high colonization of roots. These results corroborate with the findings of Sastry and Johri (1999) reporting no relationship between AMF spore number and colonization of root. AMF colonization was not detected in non mycorrhizal families however efficient AMF spores were isolated from their rhizosphere soils. All the members of non–mycorrhizal families lack hyphal infection except for *B. campestris*, *B. oleracea* var. *botrytis*, and *B. rapa* which showed 2–11% colonization. However, colonization in non–mycorrhizal plants has also been witnessed by Poveda et al. (2019) and Adekanmbi and Adewole (2019). This may be due to the intermingling of host plants roots with other mycorrhizal plant grown in the vicinity or it is plausible that crop rotation with mycorrhizal plant may have influenced sporulation in the rhizosphere of non–mycorrhizal plants. But whether an efficient symbiosis capable of benefiting the host plant is formed or not is not known because of the absence of vesicles and arbuscules in these plants. As per the studies of Wang et al. (2024) th[e topsoil](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/topsoil) intensify interactions amid root AMF by enhancing competitive relationships.

The data revealed uneven distribution of AMF species diversity that was affected by sampled location, land use type and its physico–chemical properties as well. All the six genera of AMF were detected which were widely distributed in the soil of different districts of Haryana. *Glomus* exhibited maximal abundance and frequency of occurrence followed by *Acaulospora*. Similarly, dominance of *Glomus* followed by *Acaulospora* has also been stated by other workers (Shukla et al., 2013; Gupta et al., 2018; Alrajhi et al., 2024). Members of Glomaraceae family reveal high ecological plasticity to occupy the more diverse habitats (Melo et al., 2020). According to Haug et al. (2019), AMF community compositions are influenced by stochastic processes and habitat filtering. This might also be due to the reason that *Glomus* and *Acaulospora* compete strongly for resources through a variety of strategies as compared to the other AMF genera to establish in the soil. The soil pH ranged from 6.10 to 7.40 i.e., slight acidic to neutral to slight alkaline and the presence of excessive AMF spores in this soil is in accordance with the inference of Jiao et al. (2011) and Parihar et al. (2019) that this pH range favour *Glomus* and *Acaulospora* sporulation and therefore *Acaulospora* was frequently witnessed in the soil of Ambala and Panchkula. Among *Glomus* species, *G. mosseae* and among *Acaulospora* species, *A. laevis* were the most preferred species by vegetable crops.

The AMF spores were isolated from the cultivated agricultural land which is prone to lot of disturbance in the form of various cultivation practices including tillage, implementation of fertilizer, pesticides etc which disturbs the growth and proliferation of AMF hypha and thus reduces the spore formation. In the present study in spite of mechanical disturbances in the cultivated land, AMF status was sufficient enough to provide benefits to the plants. Contrary to this Schalamuk et al. (2006) documented that tillage and fertilization did not affect AMF biodiversity. Interestingly a large number of AMF spores were encountered from the studied site, with highest number in slightly acidic soil of Panchkula, Ambala, Kurukshetra and Gurgaon which were comparatively beneficial for AMF survival confirmed by the frequent occurrence of AMF compared to that of Rewari, Jind and Sirsa that harbour least AMF spore density. This is in accordance with the view of Dessai and Rodrigues (2012) that the soil pH range from acidic to neutral inhabits a more AMF species number as compared to the neutral to slight alkaline soil of other regions and suggest that the soil AMF community can adapt to different environmental conditions and host type. Thorough microscopic investigation of the plant root segments showed the presence of arbuscules, vesicles, hyphal infection which was much toward Arum– type, but in some crops AMF hyphal coils were also seen which belongs to Paris–type. The variations in root colonization are thought to be linked to soil properties and AMF communities (Han et al., 2019).

Conclusion

Vegetable crops are recognized for their pronounced reliance on the existence of symbiotic fungal endophytes to grow, establish and produce yield. The study demonstrates that tomato crops exhibit the highest AMF colonization, which suggests their significant potential for enhancing nutrient uptake efficiency in sustainable vegetable production systems. AMF association with vegetable crops of Haryana was never analyzed before and such recommendations would provide direction for further studies The finding of the present investigation can be pathway for researcher to make AMF formulation to be used in vegetable production system. The outcomes of this inquiry pave the way for researchers to formulate AMF preparations tailored for use in vegetable production systems. The findings of the present investigation highlight the importance of AMF inoculation in vegetable cropping systems to improve nutrient use efficiency, potentially reducing the

dependence on chemical fertilizers and contributing to sustainable agriculture. Furthermore, the practical agricultural implications of this study are to aids researcher in comprehending the diversity and composition of AMF in conjunction with vegetable crops, a fundamental aspect in grasping these crops' dependency on AMF. The regional specific survey of confined number of vegetable species may limit the generalizability of the findings to other agro-ecological zones. Future research should focus on field level validation of AMF inoculation and its long-term effects on soil health and crop productivity under different environmental conditions.

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