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Impact of chemical properties of soil on spore density, colonization, and distribution of native arbuscular mycorrhizal fungi associated with *Capsicum annuum* L.

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ABSTRACT

This study is designed to investigate the impact of chemical properties of soil on spore density, colonization, and distribution of native arbuscular mycorrhizal (AM) fungal species associated with chillies (*Capsicum annuum* L.) from six sites in Phaltan tehsil of Satara District, Maharashtra, India. The AM fungi spore density and root colonization were positively correlated with pH (r = 0.470 and r = 0.246, respectively), organic carbon, N, Zn, Cu, and free lime, while they were negatively correlated with P (r = −0.025 and r = −0.148, respectively), K, and Na. The soil EC (r = 0.346 and r = −0.064, respectively), Fe, and Mn were positively correlated with spore density and were negatively correlated with root colonization. Out of the 52 species of native AM fungi identified, *Glomus* was the most frequently (55%) occurring genus with 29 species, followed by *Acaulospora* with 13 species and *Scutellospora* with 6 species. *Gigaspora* (5.7%) and *Entrophospora* (1.9%) were the least occurring genera with three and one species, respectively. The influence of soil factors on the occurrence and distribution of native AM fungi was also studied from the six selected sites. Our findings highlight the relationship between soil nutrients and AM fungi, and hence provide insight into the potential use of the combination of native species of AM fungi for the cultivation of chillies and other crops.

1. INTRODUCTION

Mycorrhizae are intricate networks of fungi belonging to phylum Glomeromycota [1], forming a symbiotic association with roots of many land plants, and creating a Wood Wide Web beneath the soil [2]. About 80% of land plants, including bryophytes, pteridophytes, and higher plants, develop mutual associations with arbuscular mycorrhizal (AM) fungi [3,4]. The AM fungi form intraradical hyphae, arbuscules, and vesicles in the cortical region of roots and extraradical hyphae and spores in the rhizosphere soil. Establishing extraradical mycelium with plant roots, AM fungi substantially increase the absorption of water and nutrients in the surface area, causing improvement in plant growth [5,6]. When associated with host plant roots, AM fungi facilitate the exchange of various macro- and microelements, like phosphorus (P), nitrogen (N), sulfur (S), potassium (K), calcium (Ca), copper (Cu), and zinc (Zn), from the soil at the cost of precious photosynthates [7–9]. Hence, AM fungi are critically important endosymbionts that have an effective role in improving plant productivity and sustainability of ecosystems [10].

A number of studies have investigated that the population, distribution, and composition of AM fungal communities in various ecosystems may be a result of environmental variations, host phenology, interspecific competition, and regional spatial dynamics [11–14]. Out of all, the soil chemical parameters act as the major contributor of dynamics of spore population, colonization, distribution, and diversity of AM fungal species [15,16], especially the availability of mineral elements [17], variations in pH [18], and electrical conductivity (EC) [19,20]. The amount of available N influences the AM fungi spore population and colonization positively [21,22], while the available P in the soil influences AM fungi colonization negatively [23,24]. In contrast to this, some experiments evidenced that high soil P supply does not always have a negative impact on AM fungi colonization [25]. Disagreements in different experiments may be due to various factors, which

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include the rate of P application, properties of soil, and climatic conditions. Soil pH [26] and organic matter [27,28] along with soil moisture content and seasonal fluctuations also influence AM fungal communities [29,30]. Moreover, AM fungal symbiosis also improves tolerance to different biotic and abiotic stresses [31–35]. Both biotic and abiotic factors affect the distribution, composition, and diversity of AM fungal communities which can act as filters for the selection of specific native species and can be used as a potential inoculum for field crops [36].

Chilli (*Capsicum annuum* L.) is the universal spice belonging to the family Solanaceae, native to Mexico and Central America [37] but cultivated throughout the world. Chilli is considered as an important crop of great commercial value and is diversely used as a vegetable, and also for culinary and spice purposes. In addition, chilli acts as an excellent source of vitamins A and C [38], and possesses various medicinal properties [39,40].

The AM fungi are considered as the best bio-inoculants and can be used as promising bio-fertilizers in sustainable crop productivity [41]. *Capsicum* spp., like many other species of plants, develops a symbiotic association with AM fungi in the soil with low nutrients availability [42]. Different field experiments carried out in Colombia [43], China [44], and India [45] showed that there is a tremendous diversity of AM fungi in the rhizosphere of *Capsicum* spp. The inoculation of chilli rhizosphere with AM fungi had a substantial difference in fruit maturity, yield, and biotic stress tolerance over the control [46]. Scientists from different parts of the world have now gained an interest in the association of native AM fungi with *C. annuum* L., with respect to P, water absorption [47], growth, production, nutrients acquisition [48], and also stress tolerance.

India is one of the leading producers and exporter of chilli and has a share of about 39.78% of the total world trade, but the productivity of chilli is low in India (1.75 t/ha) when compared to other countries, like Cape Verde, Jamaica, and Morocco, where the yield levels are higher than 10 t/ha [49]. Hence, it is important to increase the productivity of chillies in India. The strategy of using native AM fungal species from soil always proved beneficial concerning plant nutrition, adaptation to stress conditions, and productivity [50]. Since native AM fungal species are physiologically and genetically adapted to the stress conditions of the target environment, their native host [51] can serve as the best inoculum for a specific crop in a specific area. The factors affecting AM fungal population, distribution, and composition need to be studied for the specific agro-climatic zone. This would enable the selection of functionally important species and their combinations which are crucial for using AM fungi as the best biofertilizer in field conditions. The selection of a suitable AM fungal species composition proved to be the best ameliorating agent of chilli productivity under appropriate soil conditions [52].

The objective of this study is to isolate and identify the native AM fungal species from the rhizosphere of the chilli crop from selected sites in Phaltan tehsil and to study the impact of various chemical parameters of soil on their distribution, colonization, and spore density under field conditions.

2. MATERIALS AND METHODS

2.1. Study Sites and Duration

This study was carried out during January to May 2017 in cultivated chilli fields in Phaltan tehsil located in Maharashtra, India. Soil samples were collected from rhizospheres after 90 days of transplanting the chilli. The maximum temperature and relative humidity recorded during the experiment was 31.8°C–41.4°C and 75.9%–95.6%, respectively. The names of villages and the location of the six selected sites in Phaltan tehsil are shown in Table 1.

2.2. Collection of Roots and Soil Samples

The rhizosphere soil and roots of chillies were collected 90 days after transplantation, during the luxuriant flowering and fruiting stage of plants from three fields of each selected site. About 1,000 g of soil sample was collected from the rhizosphere of randomly selected plants of each field, from 15 to 30 cm depth, and were filled in polythene bags. These samples with three replicates were brought to the laboratory and stored at 5°C–10°C. Each replicate of soil sample was analyzed for chemical characteristics, like pH, EC, major, and minor elements. The soil samples were also used for the isolation, quantification, and identification of AM fungal spores. The fine roots of chilli plants were collected, rinsed with tap water, and used to investigate the percentage root colonization. The remaining roots were fixed in formalin–acetic acid–alcohol for future studies.

2.3. Estimation of Soil Chemical Properties

A portion of soil samples collected from each site was subjected to analysis of chemical properties. Soil analysis was carried out to quantify the pH, EC, organic carbon (OC, %), major elements (N, P, and K), and trace elements (sodium, free lime, Fe, Mn, Zn, and Cu). The pH of the soil was measured using a pH meter. The Electrical conductivity (dS/m) is a measure of the concentration of miscible salts in the soil was determined using conductivity meter in 1:5 (W/V) soil water suspensions at 25°C. OC was estimated using the chromic acid titration method [53]. The Kjeldahl method was used to estimate the available N content using alkaline permanganate [54]. Available P in the soil was determined by Olsen's method by extraction with sodium bicarbonate using a spectrophotometer [55]. Total exchangeable K was determined by the ammonium acetate method [56] using a flame photometer. Sodium concentration was also determined by using a flame photometer. The ethylene diamine tetra acetic acid titration method was employed to estimate free lime [57]. Fe, Mn, Zn, and Cu were estimated by acid digestion of the soil method [58].

2.4. Isolation and Estimation of AM Fungal Spore Density

The AM fungal spore density was analyzed from 100 g of rhizosphere soil by using wet sieving and decanting method [59]. The composite soil sample was used in three replicates for isolation of spores. About 100 g soil was taken from each replicate, mixed thoroughly in 1,000 ml of water, and after some time a supernatant was poured through the stacked sieves. Different sized sieves were used in a stack of 250, 210, 150, and 75 µm from top to bottom.

The spores were recovered on Whatman filter paper No. 1 and quantification was carried out using Leica EZ4 stereo-microscope. Distinguished spores/sporocarps were picked up to make slides using polyvinyl alcohol lactoglycerol (PVLG) as the mountant. The total spore count was carried out using Leica EZ4 stereo-microscope.

2.5. Identification of Native AM Fungal Species

Intact spores and sporocarps were mounted in PVLG and were identified based on their morphology using taxonomic keys, such as color, size, shape, hyphal attachment, bulbous suspensor, wall structure, number of wall layers, thickness of walls, etc. Spores were photographed and their morphological characters were studied using the Leica ICC50E Microscope with a high-definition digital camera and Leica imaging software. The AM fungal spores were identified using the Manual for Identification of vesicular arbuscular mycorrhiza Fungi [61].

2.6. Estimation of Percentage Root Colonization

Root staining and clearing method was used to prepare roots for the assessment of percentage root colonization [61]. Roots were washed thoroughly to remove soil particles and treated with 10% potassium hydroxide solution for 1 hour in a hot water bath. Then, they were washed with tap water and further treated with 2% HCl solution for 5 minutes. The acidified roots were stained with 0.05% trypan blue in lactic acid for 10–15 minutes in a hot water bath. Afterward, the roots were destained with lactic acid and observed under a compound microscope to study their fungal characteristics, like intraradical hyphae, vesicles, and arbuscules. The percentage root colonization was determined by slide count and gridline intersect method [62] using the following formula:

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

2.7. Statistical Analysis

Pearson's correlation coefficients of the different chemical parameters of soil versus AM fungal spore density and root colonization associated with chilli plants were calculated using the Statistical Package for the Social Sciences version 20.

3. RESULTS AND DISCUSSION

This study was carried out at six different sites selected in Phaltan tehsil. The aim was to study the impact of chemical properties of soil on spore density, percentage root colonization, distribution, and composition of AM fungi associated with chilli.

3.1. Chemical Properties of Soil

Means along with the standard deviation (SD) of the chemical properties of soil samples are presented in Table 2. The mean values of soil pH vary from low (7.81) to high (8.71), respectively, in L4 and L3 sites. Similar results were obtained for L4 and L3 sites

Table 1: Names of villages and location of six selected sites in Phaltan tehsil.

Sites	L1	L2	L3	L4	L5	L6
Site name	Chaudharwadi	Sangavi	Dhumalwadi	Mirewadi	Andrud	Hingangaon
Location	N.18˚.01.748′, E.074˚24.806′	N.18˚.02.254′, E.074˚29.225′	N.17˚.52.888′, E.074˚28.560′	N.18˚.06.596′, E.074˚45.376′	N.17˚.54.366′, E.074˚36.942′	N.17˚.53.232′, E.074˚29.476′

Table 2: Chemical properties of soil, spore density, and percentage root colonization associated with chilli rhizospheres in six selected sites in Phaltan tehsil.

S. No	Parameters	L1	L2	L3	L4	L5	L6
1	pH	8.28 ± 0.61	7.93 ± 0.09	8.71 ± 0.18	7.81 ± 0.48	7.89 ± 0.68	8.05 ± 0.62
2	EC (dS/m)	2.57 ± 0.44	2.42 ± 0.19	2.87 ± 0.14	2.23 ± 0.38	2.43 ± 0.09	2.39 ± 0.12
3	OC (%)	0.81 ± 0.27	0.8 ± 0.02	0.89 ± 0.52	0.56 ± 0.3	0.68 ± 0.19	0.69 ± 0.25
4	N (Kg/ha)	192.33 ± 4.62	185.67 ± 79.78	202.33 ± 57.74	128.9 ± 60.39	136 ± 21.07	166.6 ± 61.1
5	P (Kg/ha)	17.7 ± 12.9	19.73 ± 6.04	15.01 ± 12.22	19.96 ± 5.68	18.09 ± 11.1	17.43 ± 9.39
6	K (Kg/ha)	233 ± 23.1	272.67 ± 79.76	158 ± 51.45	289.33 ± 81	268.67 ± 48.05	248 ± 18.36
7	Na (mg/lit)	2.55 ± 0.99	3.18 ± 1.16	2.48 ± 0.59	4.34 ± 0.42	3.63 ± 0.81	2.86 ± 0.9
8	Free lime (%)	13.3 ± 6.43	12.19 ± 4.13	15.04 ± 0.81	8.58 ± 1.05	8.77 ± 4.26	10.64 ± 0.55
9	Fe (ppm)	0.52 ± 0.13	0.47 ± 0.14	0.45 ± 0.04	0.48 ± 0.11	0.48 ± 0.07	0.51 ± 0.07
10	Mn (ppm)	0.38 ± 0.33	0.22 ± 0.18	0.2 ± 0.16	0.21 ± 0.15	0.22 ± 0.1	0.33 ± 0.16
11	Zn (ppm)	0.12 ± 0.07	0.17 ± 0.14	0.25 ± 0.1	0.08 ± 0.05	0.12 ± 0.09	0.25 ± 0.13
12	Cu (ppm)	0.16 ± 0.06	0.21 ± 0.04	0.32 ± 0.07	0.14 ± 0.07	0.16 ± 0.07	0.23 ± 0.03
13	Spore density	683 ± 10.81	693 ± 3.61	874.67 ± 2.52	330.67 ± 3.51	417.33 ± 5.51	733.67 ± 3.51
14	% Root colonization	85 ± 3	92 ± 3	96 ± 2	84 ± 3	84 ± 3	96 ± 3

Data are presented as mean ± SD. The means were obtained from three replicates (n = 3). EC = electrical conductivity; OC = organic carbon; N = available nitrogen; P = available phosphorous; K = available potassium; Na = sodium; Fe = ferrous; Mn = manganese; Zn = zinc; and Cu = copper.

concerning EC (2.23 and 2.87 dS/m, respectively). A high amount of OC (0.89%) was recorded at the L3 site and a low amount (0.56%) was recorded at the L4 site. The highest concentrations of available N (202.33 kg/ha), available K (289.33 kg/ha), and available P (19.96 kg/ha) were recorded at L3, L4, and L4 sites, respectively, while the lowest concentrations of available N (128.9 kg/ha), available K (158 kg/ha) and available P (15.01 kg/ha) were recorded at L4, L3, and L3 sites, respectively.

3.2. Estimation of Spore Density and Root Colonization

Maximum spore density per 100 g soil (874.67) and percentage root colonization (96%) were reported at the L3 site, while minimum spore density per 100 g soil was reported at L4 (330.67) and L5 (417.33) sites, and percentage root colonization (84%) was reported at L4 and L5 sites. The intraradical hyphae, vesicles, and arbuscules occurred in the cortical region of roots which represents a good amount of colonization (Plate 2).

3.3. Correlation Analysis of Chemical Properties of Soil and AM Fungal Population

3.3.1. Correlation of pH and EC with AM fungi

In this study, AM colonization and spore density showed a positive correlation ($r = 0.246$ and $r = 0.47$ respectively) with the pH value of soil samples (Table 3). Maximum mean spore density per 100 g soil (874.67) of AM fungi and mean percentage root colonization (96%) were recorded with the highest mean pH value (8.71) at site L3 (Table 2). These findings are consistent with the previous study [63]. The occurrence of AM fungi in extremely alkaline soils with pH values up to 11 was also reported [64]. The L4 site had the lowest mean pH value (pH 7.81) and also the lowest mean spore density (330.67) and root colonization (84%) (Table 2).

It was revealed from this study that spore density per 100 g soil (874.67) and root colonization (96%) appeared to be higher in the soils with a higher EC value (2.87; Table 2). The higher mean spore count at higher EC values was reported by several researchers [65,66]. Positive Pearson's coefficient of correlation ($r = 0.346$) was found between soil EC and spore density. Many earlier studies obtained a higher spore population with higher EC values of soil, which is also called as saline soil [67]. The reason behind this maybe the strategy of mycorrhiza to produce a maximum number of spores to withstand unfavorable conditions like salinity [68]. In contrast to spore density, the AM fungal colonization of chilli roots observed a negative correlation ($r = -0.064$) with the EC of soil. These results are in agreement with various investigations [69–71]. In saline soil environments mycorrhiza may decrease or stop the spore germination and hence new colonization does not take place due to unfavourable conditions.

3.3.2. Correlation of macro- and micro-elements with AM fungi

Correlation analysis was carried out between chemical properties of soil and spore density and root colonization of AM fungi (Table 3). Pearson's correlation coefficient ranged from $r = +1$ to $r = -1$, representing a positive or negative relationship between the composition of soil nutrients, AM colonization, and spore density.

The results of this study shows a strong positive correlation of Zn ($r = 0.421$); a moderate positive correlation of available N, free lime ($r = 0.266$), Fe, Mn, and Cu; and a least positive correlation of OC ($r = 0.075$) with spore density from rhizosphere soil of all six studied sites. Whereas available K ($r = -0.239$), available P ($r = -0.025$), and Na ($r = -0.030$) were negatively correlated with spore density.

Percentage root colonization of AMF showed a strong positive correlation with pH, free lime, Zn ($r = 0.421$) and Cu; moderate correlation with OC, available N ($r = 0.178$); and a negative correlation with EC, available P ($r = -0.148$), available K ($r = -0.090$), Na ($r = -0.146$), Fe ($r = -0.047$), and Mn ($r = -0.070$). Similar results were previously obtained in chilli plants [72].

Our results of a positive correlation between available N, Zn, free lime, Cu, spore density and root colonization corroborate with other studies [73,74]. Some scientists observed a positive

Table 3: Correlation analysis between the chemical properties of soil and the AM fungal population.

S. No.	Parameters	L1	L2	L3	L4	L5	L6	Spore density	% Root colonization
1	pH	8.28 ± 0.61	7.93 ± 0.09	8.71 ± 0.18	7.81 ± 0.48	7.89 ± 0.68	8.05 ± 0.62	0.470	0.246
2	EC (dS/m)	2.57 ± 0.44	2.42 ± 0.19	2.87 ± 0.14	2.23 ± 0.38	2.43 ± 0.09	2.39 ± 0.12	0.346	-0.064
3	OC (%)	0.81 ± 0.27	0.8 ± 0.02	0.89 ± 0.52	0.56 ± 0.3	0.68 ± 0.19	0.69 ± 0.25	0.075	0.221
4	N (Kg/ha)	192.33 ± 4.62	185.67 ± 79.78	202.33 ± 57.74	128.9 ± 60.39	136 ± 21.07	166.6 ± 61.1	0.023	0.178
5	P (Kg/ha)	17.7 ± 12.9	19.73 ± 6.04	15.01 ± 12.22	19.96 ± 5.68	18.09 ± 11.1	17.43 ± 9.39	-0.025	-0.148
6	K (Kg/ha)	233 ± 23.1	272.67 ± 79.76	158 ± 51.45	289.33 ± 81	268.67 ± 48.05	248 ± 18.36	-0.239	-0.090
7	Na (mg/lit)	2.55 ± 0.99	3.18 ± 1.16	2.48 ± 0.59	4.34 ± 0.42	3.63 ± 0.81	2.86 ± 0.9	-0.030	-0.146
8	Free lime (%)	13.3 ± 6.43	12.19 ± 4.13	15.04 ± 0.81	8.58 ± 1.05	8.77 ± 4.26	10.64 ± 0.55	0.266	0.340
9	Fe (ppm)	0.52 ± 0.13	0.47 ± 0.14	0.45 ± 0.04	0.48 ± 0.11	0.48 ± 0.07	0.51 ± 0.07	0.011	-0.047
10	Mn (ppm)	0.38 ± 0.33	0.22 ± 0.18	0.2 ± 0.16	0.21 ± 0.15	0.22 ± 0.1	0.33 ± 0.16	0.104	-0.070
11	Zn (ppm)	0.12 ± 0.07	0.17 ± 0.14	0.25 ± 0.1	0.08 ± 0.05	0.12 ± 0.09	0.25 ± 0.13	0.377	0.421
12	Cu (ppm)	0.16 ± 0.06	0.21 ± 0.04	0.32 ± 0.07	0.14 ± 0.07	0.16 ± 0.07	0.23 ± 0.03	0.264	0.405

Table 4: Number and FO of species of AM fungi reported from six selected sites in Phaltan tehsil.

S. No.	Name of genus and species	L-1	L-2	L-3	L-4	L-5	L-6	FO (%)
1.	*Glomus aggregatum* Schenck and Smith	−	−	+	+	−	+	50
2.	*Glomus albidum* Walker and Rhodes	+	−	−	−	+	+	50
3.	*Glomus ambisporum* Smith and Schenck	−	−	+	−	−	−	16.6
4.	*Glomus arborense* McGee	−	+	−	−	−	−	16.6
5.	*Glomus australe* (Berkeley) Berch	−	−	−	−	−	+	16.6
6.	*Glomus boreale* (Thaxter) Trappe and Gerdemann	−	−	−	−	−	+	16.6
7.	*Glomus botryoides* Rothwell and Victor	−	+	−	+	+	+	66.6
8.	*Glomus callosum* Sieverding	−	−	−	+	−	−	16.6
9.	*Glomus cerebriforme* McGee	+	−	−	−	−	−	16.6
10.	*Glomus claroides* Schenck and Smith	−	−	−	−	+	+	33.3
11.	*Glomus clarum* Nicolson and Schenck	−	−	−	−	+	+	33.3
12.	*Glomus constrictum* Trappe	−	−	+	−	−	+	33.3
13.	*Glomus delhiense* Mukerji, Bhattacharjee and Tewari	−	−	+	−	+	+	50
14.	*Glomus deserticola* Trappe, Bloss and Menge	−	−	−	−	+	−	16.6
15.	*Glomus dimorphicum* Boyetchko and Tewari	−	−	+	−	−	−	16.6
16.	*Glomus etunicatum* Becker and Gerdemann	−	−	−	−	−	+	16.6
17.	*Glomus fasciculatum* (Thaxter) Gerdmann& Trappe emend. Walker and Koske	−	−	−	−	+	−	16.6
18.	*Glomus fistulosum* Skou and Jakobsen	+	−	+	+	−	−	50
19.	*Glomus flavisporum* (Lange & Lund) Trappe & Gerdmann	−	−	+	−	−	−	16.6
20.	*Glomus fragilistratum* Skou & Jakobsen	+	−	−	−	−	−	16.6
21.	*Glomus geosporum* (Nicolson & Gerdmann) Walker	−	−	−	+	−	−	16.6
22.	*Glomus gerdemannii* Rose, Daniels & Trappe	−	−	−	+	−	−	16.6
23.	*Glomus globiferum* Koske and Walker	−	+	+	−	−	−	33.3
24.	*Glomus heterosporum* Smith and Schenck	+	−	−	−	−	−	16.6
25.	*Glomus intraradix* Schenck and Smith	−	−	+	−	−	−	16.6
26.	*Glomus leptotichum* Schenck and Smith	+	−	−	−	−	−	16.6
27.	*Glomus mosseae* (Nicolson & Gerdmann)	−	+	+	−	+	+	66.6
28.	*Glomus multicaule* Gerdmann and Bakshi	+	−	−	−	−	−	16.6
29.	*Glomus radiatum* (Thaxter) Trappe & Gerdmann	−	−	−	−	+	−	16.6
30.	*Acaulospora appendicula* Spain, Sieverding & Schenck	+	−	−	−	−	+	33.3
31.	*Acaulospora delicata* Walker, Pfeiffer and Bloss	−	−	+	+	+	−	50
32.	*Acaulospora denticulata* Sieverding& Toro	−	−	+	−	−	−	16.6
33.	*Acaulospora elegans* Trappe and Gerdemann	−	+	−	−	−	−	16.6
34.	*Acaulospora foveata* Trappe and Janos	−	+	−	−	+	−	33.3
35.	*Acaulospora lacunosa* Morton	−	+	−	−	−	−	16.6
36.	*Acaulospora laevis* Gerdemann and Trappe	+	+	+	−	+	−	66.6
37.	*Acaulospora nicolsonii* Walker, Reed and Sanders	−	−	−	+	−	−	16.6
38.	*Acaulospora scrobiculata* Trappe	+	−	−	−	−	−	16.6
39.	*Acaulospora spinosa* Walker and Trappe	−	+	−	+	−	−	33.3
40.	*Acaulospora splendida* Sieverding, Chaveri and Rojas	+	−	−	−	−	−	16.6
41.	*Acaulospora sporocarpa* Berch	+	−	−	−	−	−	16.6
42.	*Acaulospora thomii* Blaszkowski	+	−	+	−	−	−	33.3
43.	*Scutellospora calospora* (Nicolson and Gerdemann) Walker and Sanders	+	+	+	−	−	−	50
44.	*Scutellospora dipapillosa* (Walker and Koske) Walker & Sanders	−	−	+	−	−	−	16.6
45.	*Scutellospora dipurpurascens* Mortan and Koske	−	−	−	+	−	−	16.6
46.	*Scutellospora heterogama* (Nicolson and Gerdemann) Walker and Sanders	+	+	+	−	−	−	50
47.	*Scutellospora minuta* (Ferrer and Herrera) Walker and Sanders	+	−	−	−	+	+	50
48.	*Scutellospora pellucida* (Nicolson & Schenck) Walker and Sanders	−	+	−	−	+	−	33.3
49.	*Gigaspora albida* Schenck and Smith	+	+	+	+	−	+	66.6
50.	*Gigaspora candida* Bhattacharjee, Mukerji, Tewari & Skoropad	−	−	−	+	−	−	16.6
51.	*Gigaspora margarita* Becker and Hall	−	−	+	−	−	−	16.6
52.	*Entrophospora infrequens* (Hall) Ames and Schneider	−	+	−	+	−	+	50
	Total number of species that occurred at each site	17	14	19	13	14	15	

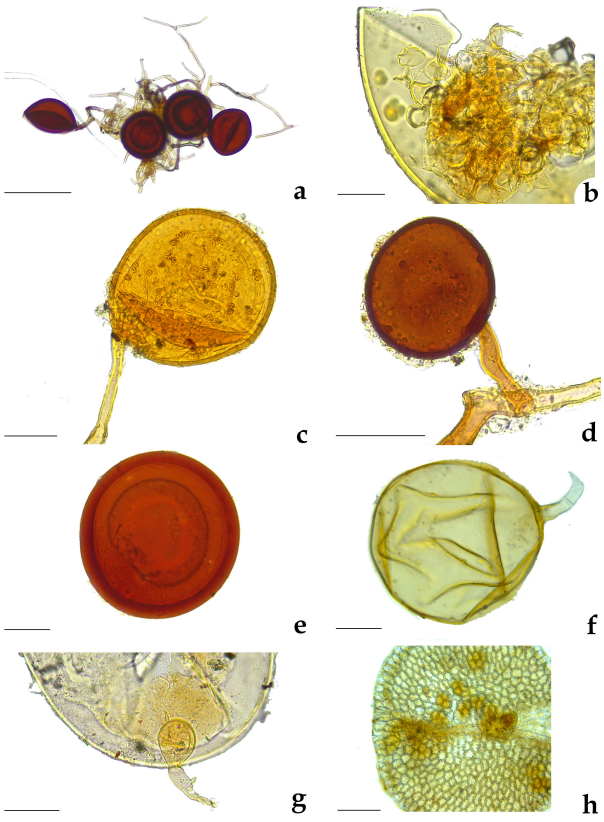

Plate 1: Diversity of spores: (a) *G. fasciculatum*; (b) *G. aggregatum* (Sporocarp); (c) *G. australe*; (d) *G. claroides*; (e) *A. appendicula*; (f) *S. calospora*; (g) *G. albida*; and (h) *E. infrequens* (Scale 50 µm).

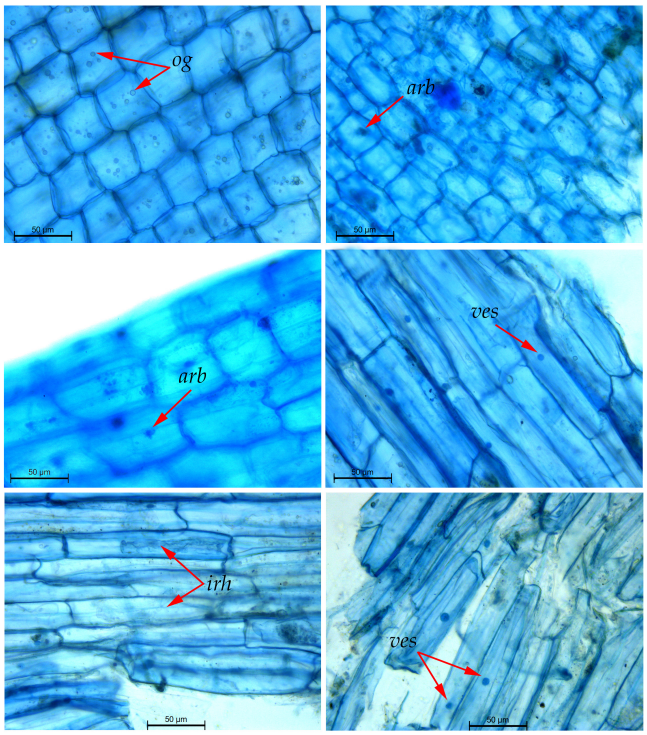

Plate 2: Percentage root colonization: og = oil globules; ves = vesicles; arb = arbuscles; irh = intraradical hyphae.

correlation between spore density, root colonization, available N, OC, and Zn in chilli crops [75]. Our results show that OC was positively correlated with AM fungal population. A higher carbon content favors the growth of AM fungi [76]. Spore density and root colonization were negatively correlated with available P content in the soil [77].

3.2.3. Taxonomy and distribution of native AM fungal species

One of the objectives of this study was to analyze the occurrence and distribution of AM fungal species associated with chilli from selected sites in Phaltan tehsil. Totally, 52 different species of AM fungi were reported from rhizosphere of chilli from six selected sites, which were identified by using morphological and other fungal diagnostic characters (Table 4, Plate 1). There was considerable diversity found in 52 species, out of which genus *Glomus* was represented by a maximum of 29 species, followed by *Acaulospora* with 13 species, *Scutellospora* with 6 species, *Gigaspora* with 3 species, and *Entrophospora* with one species. The frequency of occurrence (FO) of each species was also calculated (Table 4). It is evident from this study that *Glomus botryoides*, *Glomus mosseae*, *Acaulospora laevis*, and *Gigaspora albida* most frequently occurred at all selected sites with the highest FO (66.6%). It was followed by 50% FO for species like *Glomus aggregatum*, *Glomus albidum*, *Glomus delhiense*, *Glomus fistulosum*, *Acaulospora delicata*, *Scutellospora calospora*, *Scutellospora heterogama*, *Scutellospora minuta*, and *Entrophospora infrequens*. The remaining genera showed moderate FO about 16.6%–33.3% in all studied sites (Table 4).

The genus *Glomus* comprised of almost 55% of FO among all genera recorded at all the studied sites (Table 5). Several workers recorded similar observations; the genus *Glomus* is distributed worldwide and is also the most commonly occurring genus in cultivated lands [78]. *Acaulospora* was the next genus with a frequent occurrence (25%), followed by *Scutellospora* (11.5%), *Gigaspora* (5.7%), and the least occurring genus was *Entrophospora* (1.9%). Similar results were obtained in case of *Glomus* and *Acaulospora* [79].

The higher values of pH, EC, OC, and N were recorded at L1 and L3 sites and lower values were recorded at L4 and L5 sites (Table 2). Positive correlation was reported between these values and spore density and root colonization of AM fungal species (Table 3). Similarly, the maximum number (19) and diversity of AM species were recorded at L3 site; moderate (17) at L1 site and the least number (13) of species were recorded at L4 site (Table 5). Therefore, it can be considered that the soil chemical characteristics affected not only spore density and percentage root colonization in this study, but also the occurrence, distribution, and diversity of native AM fungal species. The specialized composition of soil chemical parameters in agriculture lands determines the specific native AM fungal species composition, which make AM fungi as a bioindicators [80]. It is evident from our study that soil chemical parameters, such as higher values of pH, EC, OC, and N can act as the driving factors of occurrence, diversity, and distribution of native AM fungal species in selected localities. Our results corroborate with previous studies [81,82].

Table 5: Distribution of different species belonging to five genera of AM fungi and FO at six selected sites.

S. No.	Name of genus	Number of species that occurred at each location						Total no. of species	FO (%) of genus with respect to no. of species
		L1	L2	L3	L4	L5	L6		
1.	*Glomus*	07	04	10	06	09	11	29	55.7
2.	*Acaulospora*	06	05	04	03	03	01	13	25
3.	*Scutellospora*	03	03	03	01	02	01	06	11.5
4.	*Gigaspora*	01	01	02	02	00	01	03	5.7
5.	*Entrophospora*	00	01	00	01	00	01	01	1.9
	Total no. of species at each site	17	14	19	13	14	15	52	

4. CONCLUSION

This study revealed the positive correlation of the AM spore density with pH and EC of the soil. The root colonization by AM fungi positively correlated with pH but not with the EC. The sites with a higher pH (alkaline) value showed higher spore density and root colonization, hence positive correlation was reported. Positive correlation of EC with spore density and negative correlation with root colonization reveal the fact that the spores being tougher and strong structures of AM fungi may increase in their number at a higher EC or saline conditions, while the low rate of root colonization may be due to the reduction in the rate of spore germination in saline soil. It is apparent from this study that OC, N, Zn, Cu, and free lime had a positive correlation, and hence there was an increase in the spore density and root colonization. The major determining factors which contributed negatively to the AM fungal population count and root colonization were available P, K, and Na. The distribution and composition of AM fungi were influenced by the chemical factors of the soil. The sites with higher values of pH, EC, N, and lower amount of P, K, and Na showed higher diversity in AM fungal species. On the contrary, a lower diversity of AM fungi was observed at sites with low pH, EC, N and a higher concentration of P, K, and Na. Altogether five genera and 52 species of AM fungi were recorded from six selected sites, out of which *Glomus* and *Acaulospora* were reported as the most frequently occurring genera, while *Scutellospora*, *Gigaspora*, and *Entrophospora* were the least occurring genera. *Glomus botryoides*, *G. mosseae*, *A. laevis*, and *G. albida* were the most frequently occurring species at all sites. The diverse composition of native AM fungi and their distribution along the studied sites was determined by soil composition. Hence, it can be concluded that the soil with alkaline pH, higher EC values (salinity), high amount of OC, N, Zn, Cu, and lower P, K, and Na concentration favors spore density, diversity, and distribution of native AM fungi associated with chilli. It is evident from this study that a combination of dominant native AM fungi can be used as the best biofertilizer for chilli cultivation.

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CONFLICT ON INTEREST

Authors declared that there are no conflicts on interest.

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