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

Komal Chandrakant Dhumal¹*, Bharat Pandharinath Shinde²

¹Department of Botany, Nowrosjee Wadia College, Pune, India.
²Principal and Head of the Department of Botany, Vidyarthi Pratishthan's Arts, Science and Commerce College, Baramati, Dist: Pune, India.

**ARTICLE INFO**

**Article history:**
Received on: May 19, 2020
Accepted on: July 09, 2020
Available online: September 14, 2020

**Key words:**
AM fungi, chilli, spore density, root colonization, distribution, chemical properties, soil.

**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

*Corresponding Author
Komal Chandrakant Dhumal, Department of Botany, Nowrosjee Wadia College, Pune, India. E-mail: komalcdhumal@gmail.com

© 2020 Dhumal and Shinde. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlike Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).
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 chilies 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 chilies 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 chloric 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 sporocarps 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 soil 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 salinity [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 arborescens 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, Blass 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 fragilisstratum Skou & Jakobsen |     |     | +   | +   |     |     | 16.6   |
| 21.    | Glomus geosporum (Nicolson & Gerdmann) Walker |     |     | +   | +   |     |     | 16.6   |
| 22.    | Glomus gerdemannii Rose, Daniels & Trappe |     |     | +   | +   |     |     | 33.3   |
| 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 multiculde 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 Blass |     |     | +     |     | +   |     | 50     |
| 32.    | Acaulospora denticulata Sieverding & Toro |     |     | +   | +   |     |     | 16.6   |
| 33.    | Acaulospora elegans Trappe and Gerdmann |     |     | +     |     |     |     | 16.6   |
| 34.    | Acaulospora foveata Trappe and Janos |     |     | +     |     |     |     | 33.3   |
| 35.    | Acaulospora lacanoi Morton |     |     | +     |     |     |     | 16.6   |
| 36.    | Acaulospora laevis Gerdmann 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 splendidula Sieverding, Chaveri and Rojas |     |     | +     |     | +   |     | 16.6   |
| 41.    | Acaulospora sponicarpa Berch |     |     | +     |     | +   |     | 16.6   |
| 42.    | Acaulospora thomii Blaszowski |     |     | +     |     | +   |     | 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 heteroagama (Nicolson and Gerdemann) Walker and Sanders |     |     | +     |     | +   |     | 16.6   |
| 47.    | Scutellospora minuta (Ferrer and Herrera) Walker and Sanders |     |     | +     |     | +   |     | 50     |
| 48.    | Scutellospora pallidula (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

Dhimal and Shinde: Impact of chemical properties of soil on spore density, colonization, and distribution of native arbuscular mycorrhizal fungi associated with *Capsicum annuum* L 2020;8(05):59-67
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 | 65                     |                            |

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.

5. ACKNOWLEDGMENTS

The authors are indebted to Mr. Yogesh Bhongale, Agriculture Officer, Phaltan Tehsil (Government of Maharashtra), for his assistance in the extensive survey and selection of sampling sites of chilli plants in Phaltan tehsil. The authors also thank the farmers of all the six selected sites who allowed the collection of soil samples from their chilli fields. They are also grateful to Mr. V. M. Bhoite, Subject Matter Specialist (Soil Science) and Krishi Vigyan Kendra (KVVK.), Baramati, for providing laboratory facilities for soil analysis. The authors extend their thanks to the Nimbkar Agriculture Research Institute (NARI), Phaltan, for providing the meteorological data of Phaltan tehsil for the year 2017.

CONFLICT ON INTEREST

Authors declared that there are no conflicts on interest.

FINANCIAL SUPPORT AND SPONSORSHIP

None.

REFERENCES

1. Schüssler A, Schwarzott D, Walker C. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 2001;105(12):1413–21; doi:10.1017/S0953756201005196.
2. Beiler KJ, Durall DM, Simard SW, Maxwell SA, KretzerAM. Architecture of the wood-wide web: Rhizopogon spp. genets link multiple Douglas-fir cohorts. New Phytol 2010;185(2):543–53; doi:10.1111/j.1469-8137.2009.03069.x.
3. Zhu XC, Song FB, Liu SQ, Liu TD, Zhou X. Arbuscular mycorrhizae improves photosynthesis and water status of Zea mays L. under drought stress. Plant Soil Environ 2012;58(4):186–91; doi:10.17221/23/2011-pse.
4. Lehnter M, Kessler M. Mycorrhizal relationships in lycophytes and ferns. Fern Gazette, British Pteridological Society, The Natural History Museum, Cromwell Road, London, UK (February 2018), 20(3), pp 101–16, 2016. Available via https://www.researchgate.net/publication/318268607.
5. Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M. Arbuscular mycorrhizal fungi act as bio-stimulants in horticultural crops. Sci Hort 2015;196:91–108; doi:10.1016/j.scienta.2015.09.002.
6. Bowles TM, Barrios-Massias FH, Carlisle EA, Cavagnaro TR, Jackson LE. Effects of arbuscular mycorrhization on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. Sci Total Environ 2016;566:1223–34; doi:10.1016/j.scitotenv.2016.05.178.
7. Porras-Soriano A, Soriano-Martín ML, Porras-Piedra A, Azcón R. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. J Plant Physiol 2009;166:1350–9; doi:10.1016/j.jplph.2009.02.010.
8. Bati CB, Santilli E, Lombardo L. Effect of arbuscular mycorrhizal fungi on growth and on micronutrient and macronutrient uptake and allocation in olive plantlets growing under high total Mn levels. Mycorrhiza 2015;25:97–108; doi:10.1007/s00572-014-0589-0.
9. Wang W, Shi J, Xie Q, Jiang Y, Yu N, Wang E. Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. Mol Plant 2017;10(9):1147–58; doi:10.1016/j.molp.2017.07.012.
10. Gianinazzi S, Golotte A, Binet MN, Van Tuinen D, Redecker D, Wipf D. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 2010;20:519–30; doi:10.1007/s00572-010-0333-3.
11. Öpik M, Moora M, Liira J, Zobel M. Composition of root colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe: arbuscular mycorrhizal fungal communities around the globe. J Ecol 2006;94:778–90.
12. Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J Ecol 2007;95:95–105.
Zhao H, Li X, Zhang Z, Zhao Y, Yang J, Zhu Y. Species diversity and drivers of arbuscular mycorrhizal fungal communities in a semi-arid mountain in China. Peer J 2017;5:e4155; doi:10.7717/peerj.4155

Melo CD, Walker C, Krüger C, Borges PVG, Luna S, Mendonça D, et al. Environmental factors driving arbuscular mycorrhizal fungal communities associated with endemic woody plant Picconia azorica on native forest of Azores. Ann Microbiol 2019;69(13):1309–27; doi:10.1007/s13213-019-01535-x

Song J, Han Y, Bai B, Jin S, He Q, Ren J. Diversity of arbuscular mycorrhizal fungi in rhizosphere soils of the Chinese medicinal herb Sophora flavescens Ait. Soil Tillage Res 2019;195:104423. doi: 10.1016/j.still.2019.104423

Smilauer P, Smilauerov M. Contrasting effects of host identity, plant community, and local species pool on the composition and colonization levels of arbuscular mycorrhizal fungal community in a temperate grassland. New Phytol 2020;225(1):461–73; doi:10.1111/nph.16112

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci USA 2010;107:2093–8.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, et al. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol 2011;190:794–804; doi:10.1111/j.1469-8137.2010.03636.x

Giri B, Kapoor R, Mukerji KG. Improved tolerance of Acacia nilotica to salt stress by arbuscular mycorrhiza Glomus fasciculatum may be partly related to elevated K/Na ratios in root and shoot tissues. Microb Ecol 2007;54:753–60.

Sheng M, Tang M, Chan H, Yang B, Zhang F, Huang Y. Influence of arbuscular mycorrhizea on photosynthesis and water status of maize plants under salt stress. Mycorrhiza 2008;18:287–96.

Egerton-Warburton LM, Johnson NC, Allen EB. Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. Ecol Monogr 2007;77:527–44.

Silvana VM, Carlos FJ, Lucia AC, Natalia A, Marta C. Colonization dynamics of arbuscular mycorrhizal fungi (AMF) in Ilex paraguariensis crops: Seasonality and influence of management practices. J King Saud Univ Sci 2020;32(1):183–8; doi:10.1016/j.jsus.2018.03.017

Bainard LD, Bainard JD, Hamel C, Gan Y. Spatial and temporal structuring of arbuscular mycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi-arid prairie agroecosystem. FEMS Microbiol Ecol 2014;88:333–44; doi:10.1111/1574-6941.12300

Nguyen TD, Cavagnaro TR, Watts-Williams SJ. The effects of soil phosphorus and zinc availability on plant responses to mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol 2013;198:546–56; doi:10.1111/nph.12169

Gosling P, Andrew M, Maude P, Hammond JP, Bending, GD. Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. New Phytol 2013;198:546–56; doi:10.1111/nph.12169

Melo CD, Luna S, Krüger C et al. Arbuscular mycorrhizal fungal community composition associated with Juniperus brevifolia in native Azorean forest. Acta Oecol 2017;79:48–61.

Torrecillas E, Alguacil MM, Roldán A, Díaz G, Montesinos-Navarro A, Torres P. Modularity reveals the tendency of arbuscular mycorrhizal fungi to interact differently with generalist and specialist plant species in gymospore soils. Appl Environ Microbiol 2014;80:5457–760; doi:10.1128/AEM.01358-14

Wang C, Gu Z, Cui H, Zhu H, Fu S, Yao Q. Differences in arbuscular mycorrhizal fungal community composition in soils of three land use types in subtropical hilly area of Southern China. PLoS One 2015;10(6):1–16; doi:10.1371/journal.pone.0130983

Deepika S, Kothama A. Soil moisture - a regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. Mycorrhiza 2015;25:67–75; doi:10.1007/s00572-014-0596-1

Shinde BP, Singh N. Effect of arbuscular mycorrhizal fungi on growth parameters of sweet corn under NaCl salinity. Int J Curr Microbiol Appl Sci 2017;6(2):1317–25; doi:10.20546/ijcmas.2017.602.149

Bhosale KS, Shinde BP. Influence of arbuscular mycorrhizal fungi on proline and chlorophyll content in Zingiber officinale Roxs grown under water stress. Indian J Fund Appl Life Sci 2011;1(3):172–6. Available via http://www.cibtech.org/ijls.htm

Shinde SK, Shinde BP, Patale SW. The alleviation of salt stress by the activity of AM fungi in growth and productivity of onion (Allium cepa L.) plant. Int J Life Sci Pharma Res 2013;3(1):11–5.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, et al. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci 2019;10:1–15; doi:10.3389/fpls.2019.01068

Salam EA, Alataar A, El-Sheikh MA. Inoculation with arbuscular mycorrhizal fungi alleviates harmful effects of drought stress on damask rose. Saudi J Biol Sci 2017;25(8):1772–80; doi:10.1016/j.sjbs.2017.10.015

Liu X, Fu ZY, Zhang B, Zhai L, Meng MJ, Lin J, et al. Effects of sulfuric, nitric, and mixed acid rain on Chinese fir sapling growth in Southern China. Ecotoxicol Environ Saf 2018;160:154–61; doi:10.1016/j.ecoenv.2018.04.071

Kivlin SN, Hawke CV, Treseder KK. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biol Biochem 2011;43:2294–303; doi:10.1016/j.soilbio.2011.07.012

Pozzobon MT, Schiffino-Wittmann MT, Bianchetti LB. Chromosome numbers in wild and semi domesticated Brazilian Cupania L. (Solanaeaceae) species: do n = 12 and n = 13 represent two evolutionary lines? Bot J Linn Soc 2008;151:259–69.

Mican KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem 2001;49:3106–12.

Yang ZH, Wang XH, Wang HP, Hu LQ, Zheng XM, Li SW. Capsaicin mediates cell death in bladder cancer T24 cells through reactive oxygen species production and mitochondrial depolarization. Urology 2010;75:335–41.

López P, Górzalezny S, Acevedo C, Alonso R, Ferrraro G. Chemical study anti-inflammatory activity of Capsicum chacoense and C. baccatum. Rev Bras Farmacogn 2012;22:455–8; doi:10.1590/s0102-695x2011005000187

Barrow, CJ. Biochear potential for countering land degradation and for improving agriculture. App Geogr 2012;34:21–8; doi:10.1016/j.aggio.2011.09.008

Davies FT, Jr. Potter JR, Linderman RG. Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. J Plant Physiol 1992;139:289–94.

Cardona G, Peña-Venegas CP, Arcos A. Ocurrencia de hongos formadores de micorriza arbuscular asociados a aji (Capsicum sp.) en la Amazonia colombiana. Agronomia Colobum 2008;26:459–70.

Hu Y, Rillig MC, Xiang D, Hao Z, Chen B. Changes of AM fungal abundance along environmental gradients in the arid and semiarid grasslands of Northern China. PLoS One 2013;8:e57593; doi:10.1371/journal.pone.0057593

Vyas M, Vyasa A. Diversity of arbuscular mycorrhizal fungi associated with rhizosphere of Capsicum annum in Western Rajasthan. Int J Plant Anim Environ Sci 2012;2:256–62.

Long Xq, Cui WD, Yong R, Feldmann F. Enhanced yield and disease tolerance of field cucumber, field pepper and potted marigold following AMF inoculation. In: F. Feldmann, Y. Kapulnik, J. Baar (eds.). Mycorrhiza works. DPG Publisher, Braunschweig, Germany, 2008.

Sharif M, Claassen N. Action mechanisms of arbuscular mycorrhizal fungi in phosphorus uptake by Capsicum annum L. Pedosphere 2011;21:502–11.
The arbuscular mycorrhizal fungus Glomus intraradices in the rhizosphere in arid and semi-arid Algerian areas. Sci Technol Environ 2016;29(2):267–72; doi:10.1058/j.scientificen.2016.00053.7

50. Malz T, Treseder KK. Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis. Restor Ecol 2015;23:625–34; doi:10.1111/rec.12231

51. Oliveira AN, Oliveira LA. Influence of edapho-climatic factors on the sporulation and colonization of arbuscular mycorrhizal fungi in two Amazonian native fruit species. Braz Achiev Biotechnol 2010;53:653–61.

52. Kim SJ, Eo JK, Lee EH, Park H, Eom AH. Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. Mycobiology 2017;45(1):20–4; doi:10.5941/MYCOTO.2017.45.1.20

53. Walkely AJ, Black IA. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci 1934;37:29–38.

54. Subbiah BV, Asija GL. A rapid procedure for determination of available nitrogen in soils. Curr Sci 1956;25:259–60.

55. Olsen SR, Cole CV, Watanabe FS, La D. Estimation of available phosphorus by extraction with sodium bicarbonate (Circular 39). USDA, Washington, DC, 1954.

56. Hanway JJ, Heidel H. Soil analysis methods as used in Iowa state college soil testing laboratory. Iowa State Coll Agric Bull 1952:57:1–31.

57. GOI. Methods manual soil testing in India. Ministry of Agriculture Government of India, New Delhi, India, pp 1–215, 2011.

58. Jackson ML. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi, India, pp 36–82, 1967.

59. Gerdemann JW, Nicolson TH. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 1963;46:235–44.

60. Schenck NC, Perez Y. Manual for identification of VA Mycorrhizal fungi. 3rd edition, Synergetic Publications, Gainesville, FL, 1990.

61. Philips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Transact Br Mycol Soc 1970;55(1):158–61.

62. Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular-arbuscular infection in roots. New Phytol 1980;84:489–500.

63. Sharif M, Moawad M. Arbuscular mycorrhizal incidence and infectivity of crops in northwest frontier province of Pakistan. World J Agric Sci 2006;2(2):123–32.

64. Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Tóth T, Biró B, Agarwal A, Treseder KK. Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis. Restor Ecol 2015;23:625–34; doi:10.1111/rec.12231

65. Oliveira AN, Oliveira LA. Influence of edapho-climatic factors on the sporulation and colonization of arbuscular mycorrhizal fungi in two Amazonian native fruit species. Braz Achiev Biotechnol 2010;53:653–61.

66. Kim SJ, Eo JK, Lee EH, Park H, Eom AH. Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. Mycobiology 2017;45(1):20–4; doi:10.5941/MYCOTO.2017.45.1.20

67. Walkely AJ, Black IA. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci 1934;37:29–38.

68. Subbiah BV, Asija GL. A rapid procedure for determination of available nitrogen in soils. Curr Sci 1956;25:259–60.

69. Olsen SR, Cole CV, Watanabe FS, La D. Estimation of available phosphorus by extraction with sodium bicarbonate (Circular 39). USDA, Washington, DC, 1954.

70. Hanway JJ, Heidel H. Soil analysis methods as used in Iowa state college soil testing laboratory. Iowa State Coll Agric Bull 1952;57:1–31.

71. GOI. Methods manual soil testing in India. Ministry of Agriculture Government of India, New Delhi, India, pp 1–215, 2011.

72. Jackson ML. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi, India, pp 36–82, 1967.