

Original Research Article

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Effect of Arbuscular Mycorrhizal Fungi on Soil Chemical Characteristics in Apple Orchards of Kashmir Himalaya

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ABSTRACT

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An attempt was made to analyze the correlation between physico-chemical factors of soil with distribution of AM fungi under temperate conditions in different apple orchards of Kashmir Himalaya. Ten villages from district Pulwama were selected for the present investigation because edaphic factors of the particular district show variable soil. The study revealed that all chemical factors viz. Organic carbon, Available nitrogen, Available Potassium and Available phosphorus are positively correlated with distribution of AM fungi.

Introduction

Arbuscular mycorrhizal fungi (AMF) are geographically ubiquitous and occur over a broad ecological range. They are commonly found in association with numerous plant species including Agricultural and Horticultural crops. Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota are considered to associate with at least 80 per cent of vascular land plants. The association of plants with AMF improves plant establishment, growth and productivity. These fungi are well known to enhance plant nutrient uptake, plant tolerance to drought and abiotic stresses and to protect them against pathogens

and nematodes. Additionally, AM fungi stabilize soils and improve soil structure through binding sand grains and aggregate formation.

The root infections by AMF consist of intercellular hyphae and vesicles together with finely branched arbuscules which develop within the host cortical cells. External mycelium attaches to the roots, ramifies into the surrounding cells and produces small vegetative spores and larger resting spores singly or in sporocarps. The plants are responsive to inoculation with mycorrhizal fungi, especially in soils which are low in fertility (Mosse, 1973; Hayman, 1982).

Material and Methods

Study area

District Pulwama is an important part of Kashmir valley with respect to the agricultural perspective and is surrounded by Srinagar in the north, Budgam and Poonch in the west and Anantnag and Shopian in the east and south side. The district is situated between 33°46′ to 33°58′ N Latitude and 74°45′ to 75°13′ E longitude with a mean elevation of about 1630 m amsl.

It contributes a total geographical area of 0.109 m ha out of which 0.02365 m ha is under agriculture and 0.0412 m ha under forest cover, rest being used for other purposes. Pulwama soils are shallow to deep, mostly loam to silty loam and silty clay to clay. The wide variations in soil characteristics are mostly associated with slope aspect. The soils are mostly subjected to moderate to severe erosion and have moderate surface stoniness at some places.

Isolation and purification of *Arbuscular mycorrhizal* spores

Isolation of AM fungal spores from the rhizospheric soil samples was done by following Wet sieving and decanting method (Gerdemann and Nicolson 1963). The spores were counted under microscope Olympus CH20i with magnification of 10×40. Spore population was then expressed in terms of number of spores per 100 gm of dry soil (Table 1). Clean and intact spores were isolated using a specially designed needle, spores were mounted with PVLG (poly vinyl alcohol+ lactic acid+ glycerol) + Melzer's Reagent and observed under microscope and photographed. Identification of spores up to generic level was based on spore size, spore colour, wall layers and hyphal attachments using the species descriptions provided by

INVAM (<http://invam.caf.wvu.edu>) and other suitable references (Schenck and Perez, 1990; Morton and Benny, 1990; Almeida and Schenck, 1990; Bentivenga and Morton, 1995; Walker and Vestberg, 1998).

Assessment of AM fungal colonization of isolated spores

The isolated spores were further purified and mass multiplied on maize. Surface sterilized healthy maize seeds, pre-germinated in Petri plates under aseptic conditions, were sown in polythene bags containing sterilized soil + sand mixture (1:2 w/w). These bags were aseptically inoculated with identical AM spores at 5 cm depth (Jackson, 1973).

The bags were kept in a greenhouse at 25±3°C and irrigated with sterile water. The plants were uprooted after 45 days. The roots were collected, washed with sterile water to remove adhering soil debris and observed for mycorrhizal infection. The infectivity was proved by noticing the presence of Hartig net, vesicles, arbuscules or hyphae of endophytes on roots.

For estimating mycorrhizal root colonization, the root samples were collected and washed carefully to remove the adhering debris. The tertiary roots were cut into small pieces of approximately 1cm length and subjected to differential staining as described by (Phillips and Hayman, 1970). The estimation of mycorrhizal infection in roots was made by visual observation (Giovannetti and Mosse, 1980). A randomly selected aliquot of stained root segments, suspended in water, was spread in a Petridish viewed under a dissecting microscope at a magnification of 10 and 40×. In case of AM colonization, root segments containing vesicles and arbuscules of endophyte and number of mycorrhizal short roots were considered infected as suggested by Beckjord *et al.*, (1984).

Per cent mycorrhizal infection = Number of infected root segments / Total number of segments examined \times 100

The data recorded during the investigation was statistically analyzed with the help of Pearson correlation (Gomez and Gomez, 1984).

Chemical parameters

The organic carbon, available nitrogen, Available phosphorus and available K of the soil were estimated by method of Walkey and Black (1934), Alkaline permagnate method (subhaiah and Asija, 1956), olsen's method Olsen *et al.*, (1954) and Flamephotometric method (Jackson, 1958) respectively.

Results and Discussion

Morphological characterization of Arbuscular mycorrhizal spores

Spore morphology and wall characteristics were considered for the identification of *Arbuscular mycorrhizal* fungi. Four types of genera viz., *Glomus*, *Acaulospora*, *Scutellospora* and *Gigaspora*, were recovered and identified. 3 to 6 unidentified spores per gram from all studied locations were tagged as unidentified spores (Table 2). The spore colour of the species of *Glomus* was of wide range.

It varied from red-brown to almost black or straw to dark orange but most was yellow brown in colour. Spores possessed globose to sub-globose shape, about 40 to 120 μ m in size. Spore wall consisted of three layers (L1, L2 and L3). Our findings corroborate with those of many other workers (Koske, 1984; Koske and Gemma, 1990). *Acaulospora* spores were present singly in the soil and develop laterally on the neck of asporiferous saccule. Spores were light orange to yellowish brown (Table 3 and Figures 1, 2, 3 and 4)

globose to sub-globose in shape and 150 to 210 μ m in diameter. These spores were triple layered with L1 which forms the spore surface light yellow to apricot yellow in colour and 0.7 to 2.0 μ m in thickness. L2 was laminate and light orange to yellowish brown, 6.8 to 7.4 μ m in thickness. L3 was laminate, hyaline, 0.8 to 1.6 μ m in thickness and usually tightly adherent to L2.

Similar observations have been reported by others also (Walker *et al.*, 2007), Sharma *et al.*, (2009). *Scutellospora* spores were with or without ornamentations. Spores consisted of a bilayered spore wall and two bilayered flexible inner walls.

Thin-walled auxillary cells with smooth to knobby surfaces were produced on hyphae in the soil near the root surface and were also reported by (Schenck and Perez, 1990) and (Morton, 2002). *Gigaspora* spore wall consisted of a permanent outer layer enclosing a laminate layer, each with different properties that distinguish species (e.g. color, thickness, etc). Our observations corroborate with those of Koske (1987) and Bentivenga and Morton (1995).

There was no evidence of any ectomycorrhizal association with apple roots, and this corroborates with the findings of Greene *et al.*, (1982). *Glomus* species was common and made up for more than 75% of total isolates followed by *Acaulospora*, *Gigaspora* and *Scutellospora*.

Dominancy of *Glomus* in the present study is in agreement with the findings of many other workers (Mridha and Dhar, 2007; Burni *et al.*, 2009; Sharma *et al.*, 2009). The predominance of *Glomus* spp. under varying soil conditions might be due to the fact that they are widely adaptable to the varied soil conditions and survive in acidic as well as in alkaline soils (Pande and Tarafdar, 2004).

Root colonization studies of *Arbuscular mycorrhizal* fungi

In the current study, the AM colonization in the apple roots from Pulwama district varied between 65.87 and 79.78% (Table 4, Figure 5, 6, 7, and 8). The results are in conformity with the Kandula *et al.*, (2006) who also observed higher colonization in the apple roots and confirmed the ubiquitous nature of AMF spores. The highest root colonization was recorded in response to the inoculation with *Glomus* spp. (79.78%) followed by *Acaulospora* species (79.56%), *Gigaspora* species (73.56%) and *Scutellospora* species (71.23%). Similar results were reported by some workers Gosal *et al.*, (2003) (Smith and Read, 2008).

Results of the present study indicate that the nutrient contents of the soils played a significant role in occurrence of different species of *Arbuscular mycorrhizal* fungi and it is evident from the Perusal of the data presented in Table 2 which revealed that AM spore population of district Pulwama was positively and significantly correlated with organic carbon ($r=0.887^{**}$). The results are in conformity with those of Lipinski *et al.*, (2003) who also reported a significant positive correlation between soil organic carbon and AM spore population.

There was a significant correlation between AM spore population and root colonization ($r=0.512^*$) in district Pulwama. Kumar *et al.*, (2013) also found a significant positive correlation between mycorrhizal spores and colonization. Yang *et al.*, (2010) found a positive correlation between and mycorrhizal colonization and spores. These results are also supported by Li *et al.*, (2009). The positive and significant correlation between AM spores and available nitrogen ($r=0.815^*$) was found which might be due to the fact that nitrogen and organic carbon are required by micro-

organisms for their special requirements and as a result high nitrogen and organic carbon in the soil increased infection and population of AM fungi. Similar results were reported by Venkatrao *et al.*, (1972).

Since the climatic conditions of the study area fall under temperate zone which are conducive to the mycorrhizal development, it is possible that concentration of such propagules may be higher (Akhter, 2005)

Moreover, influence of apple roots through their exudates cannot be ruled out which needs further studies.

Chemical analysis of soil

Organic carbon

Perusal of the data presented in Table 5 indicates that organic carbon content of soils of identified villages ranged between 1.68% (Shiekhar) and 1.96% (Pinglin) with an average of 1.80%. The organic carbon content of Pinglin soil was significantly more than the soils of other villages except for Rajpora and Shiekhar which was statistically at par. Najar (2002) found that organic carbon content varied from 1.4 to 2.4, 0.9 to 1.8 and 0.5 to 1.2 in the high altitude and valley basin orchard soils of Kashmir. Wani *et al.*, (2010) reported organic carbon content of 1.1 to 2.10% in the soils of Himalayan region. The variation in the organic carbon content may be due to variation in the application of organic manures and the population of the soil microorganisms. As a food source for soil fauna and flora, soil organic carbon plays an important role in the soil food web by controlling the number and types of soil inhabitants which serve important functions such as nutrient cycling and availability, assisting root growth and plant nutrient uptake, creating burrows and even suppressing crop diseases. These findings are supported by Bulluck *et al.*, (2002).

Table.1 Mean Spore count per gram of rhizosphere soil of District Pulwama

Villages	Spore population/g
Rajpura	7
Shadimarg	12
Nikas	7
Drubgam	7
Shiekar	8
Tikkin	10
Sunsomil	8
Pinglin	12
Gungoo	8
Puhoo	8
Mean	8.7

Table.2 Isolation of *Arbuscular mycorrhizal* spores from rhizospheric soil of apple from different locations of District Pulwama

Locations	Spore count per gram of soil (Identified Genera)				
	<i>Acaulospora</i>	<i>Scutellospora</i>	<i>Gigaspora</i>	<i>Glomus</i>	Unidentified Genera
Rajpura	-	-	1	2	4
Shadimarg	2	-	3	3	4
Nikas	1	2	-	-	4
Drubgam	-	1	1	2	3
Shiekar	2	-	1	-	5
Tikkin	3	-	-	3	4
Sunsomil	-	-	2	2	4
Pinglin	3	2	1	-	6
Gungoo	-	1	-	4	3
Puhoo	3	-	-	-	5

Table.3 Morphological features of isolated genera of AM fungi

Genera	Spore size (µm diameter)	Spore shape	Spore colour	Spore wall	Hyphal colour
<i>Acaulospora</i>	115-170	Globose to sub globose	Yellow brown to dark brown	Three layered (L1.L2 and L3)	Grey white
<i>Gigaspora</i>	200-300	Globose to sub globose	White to cream usually a rose pink tint.	Bilayered layered (L1 and L2)	Orange brown
<i>Scutellospora</i>	100-170	Sub globose to ellipsoid to oblong	Cream to yellow or pale orange brown to dark orange brown	Bilayered spore wall (L1 and L2)	Hyaline to orange white.
<i>Glomus</i>	40 – 120	Globose to ellipsoid	red brown to almost black most are yellow brown	three layered (L1,L2 and L3)	Hyaline to yellowish.

Locations	Genera	Root colonization (%)
Rajpora	<i>Gigaspora</i> sp.	68.23
	<i>Glomus</i> sp.	72.05
Shadimarg	<i>Acaulospora</i> sp.	65.87
	<i>Gigaspora</i> sp.	70.03
	<i>Glomus</i> sp.	72.13
Nikas	<i>Scutellospora</i> sp.	69.09
	<i>Acaulospora</i> sp.	70.05
Drubgam	<i>Scutellospora</i> sp.	71.23
	<i>Glomus</i> sp.	76.67
	<i>Gigaspora</i> sp.	69.08
Shiekar	<i>Gigaspora</i> sp.	73.56
	<i>Acaulospora</i> sp.	68.67
Tikkin	<i>Acaulospora</i> sp.	72.13
	<i>Glomus</i> sp.	68.78
Sunsomil	<i>Gigaspora</i> sp.	67.67
	<i>Glomus</i> sp.	79.55
Pinglin	<i>Acaulospora</i> sp.	79.56
	<i>Gigaspora</i> sp.	67.80
	<i>Scutellospora</i> sp.	65.87
Gungoo	<i>Scutellospora</i> sp.	69.77
	<i>Glomus</i> sp.	79.78
Puhoo	<i>Acaulospora</i> sp.	76.86

Villages	Or. Carbon (%)	Av. Nitrogen (kg/ha)	Av. Phosphorus (kg/ha)	Av. Sulphur (Kg/ha)	Av. Potassium (kg/ha)
Rajpura	1.74	352.23	16.21	11.23	186.01
Shadimarg	1.77	359.04	17.63	11.92	188.20
Nikas	1.93	370.01	16.43	12.51	186.11
Drubgam	1.79	365.34	17.34	12.01	188.23
Shiekar	1.68	343.12	17.98	11.61	183.65
Tikkin	1.83	360.05	17.23	12.13	188.06
Sunsomil	1.74	351.23	17.01	11.61	180.13
Pinglin	1.96	385.12	17.63	12.90	189.12
Gungoo	1.82	358.07	17.60	12.02	188.14
Puhoo	1.75	356.12	17.62	11.74	188.02
Mean	1.801	360.033	17.268	11.968	186.567
CD (p≤0.05)	0.069	2.589	0.306	0.702	NS
CV	2.22	8.416	1.025	3.428	1.679

Table.6 Correlation between spore population and other studied parameters of District Pulwama

Parameters	Spore population
Spore population	1
Organic carbon	0.887**
Available Nitrogen	0.815*
Available Phosphorus	0.797
Available Sulphur	0.910
Available Potassium	0.614*
Root colonization	0.512*

***. Correlation is significant at the 0.05 level**
****.** Correlation is significant at the 0.01 level

Table.7 Correlation between available nutrients of representative samples in District Pulwama

Nutrients	Organic Carbon	Available Nitrogen	Available Phosphorus	Available Sulphur	Available Potassium
Organic carbon	1	0.931**	0.514	0.923**	0.784*
Available Nitrogen	0.931**	1	0.575	0.803**	0.583
Available Phosphorus	0.514	0.575	1	0.406	0.530
Available Sulphur	0.923**	0.803**	0.406	1	0.493
Available Potassium	0.784*	0.583	0.530	0.493	1

***. Correlation is significant at the 0.05**
****.** Correlation is significant at the 0.01 level

Study area

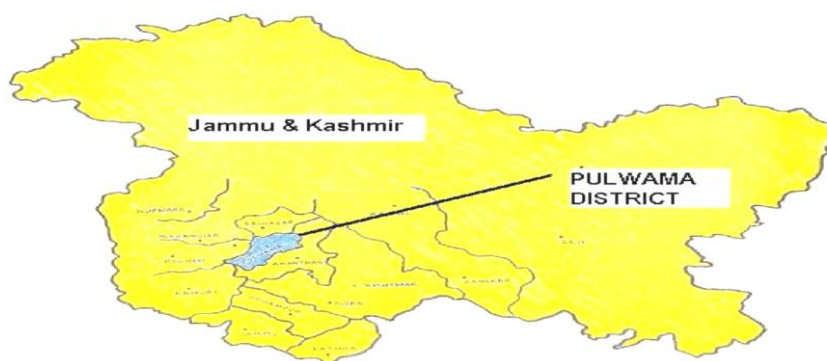


Fig.1 Spores of *Acaulospora*

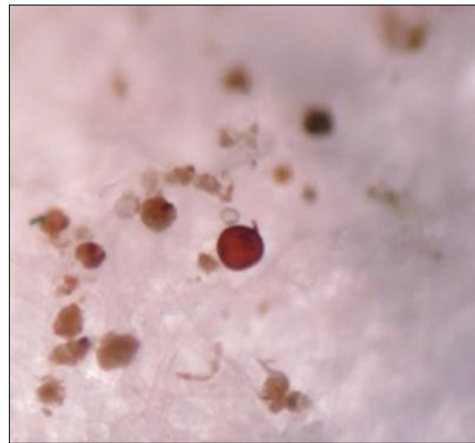


Fig.2 Spore of *Glomus*

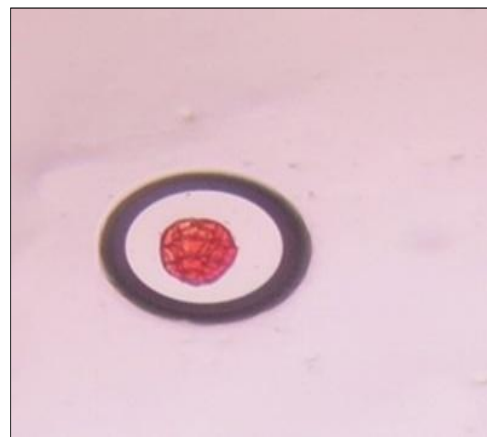
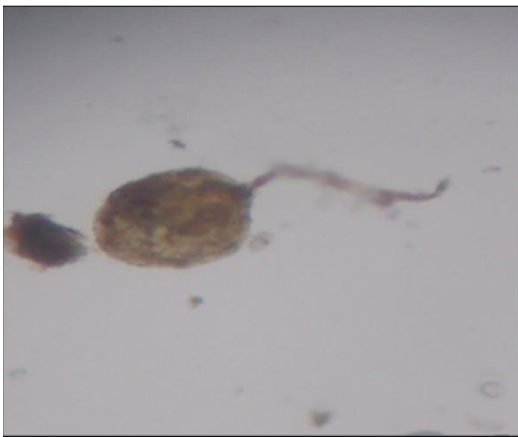


Fig.3 Spores of *Gigaspora*



Fig.4 Spores of *Scutellospora*

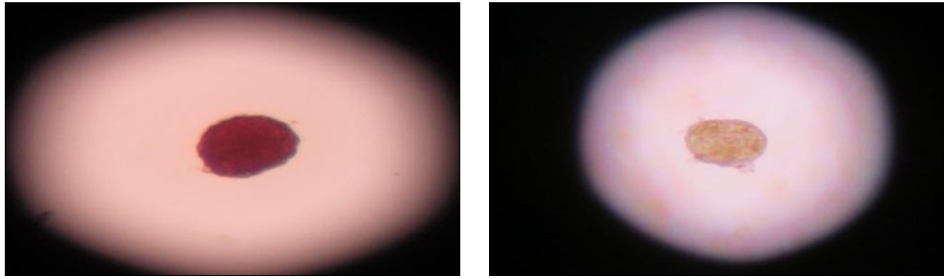


Fig.5 Root colonization of the genus *Acaulospora*

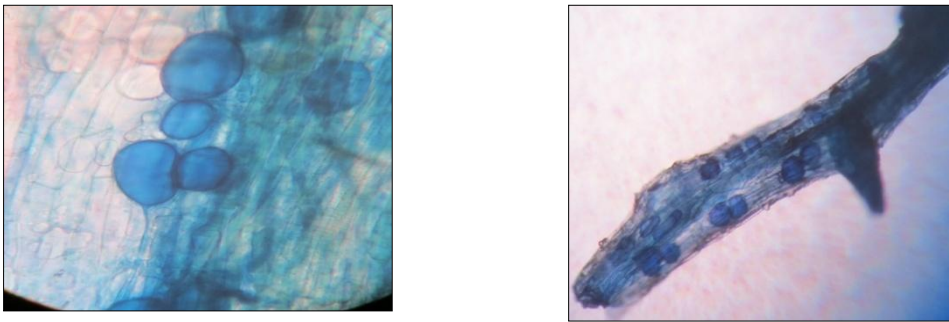


Fig.6 Root colonisation of the genus *Scutellospora*

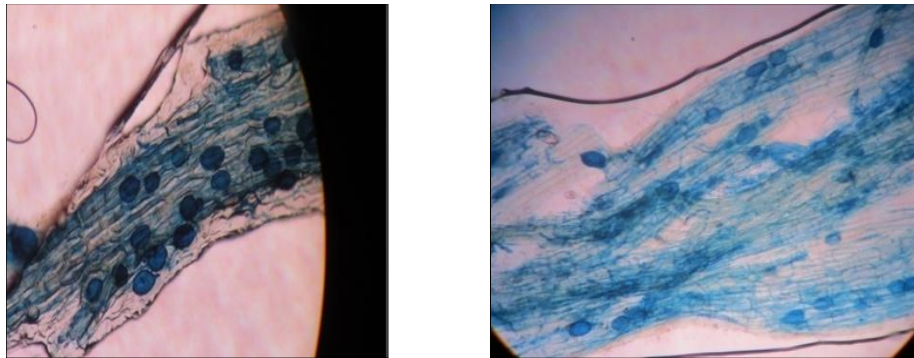


Fig.7 Root colonisation of the genus *Glomus*

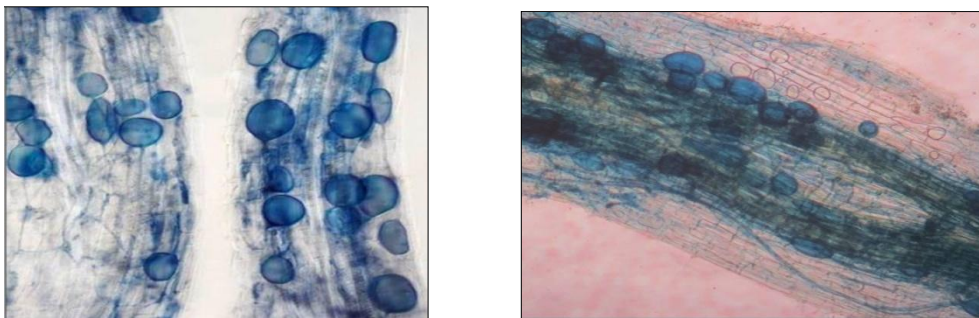
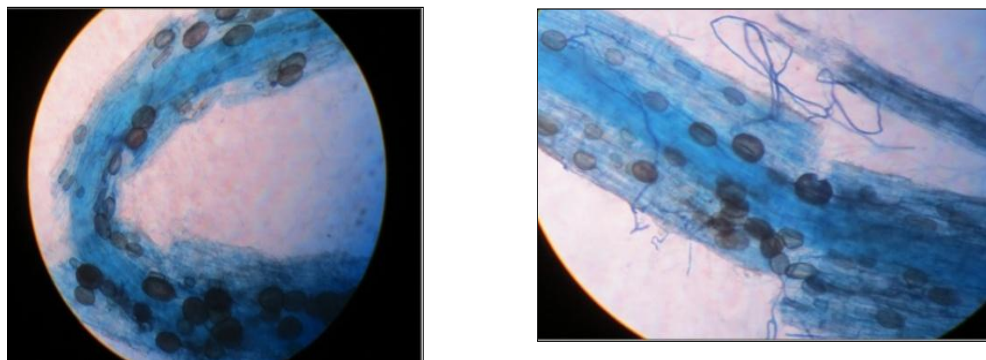


Fig.8 Root colonisation of the genus *Gigaspora*



Available nitrogen

Available nitrogen content of the soils ranged from 343.12 kg ha⁻¹ (Shiekhar) and 385.12 kg ha⁻¹ (Pinglin) with an average value of 360.03 kg ha⁻¹. The available nitrogen content of Pinglin soil was significantly more than soils of other villages. However, available nitrogen of soils from Shadimarg and Gungoo were statistically at par. This is supported by the findings of Dar (1996). Najar (2002) also observed the available nitrogen content in the range of 200 to 350 kg/ha. Bhat (2001) while studying the apple orchard soils of north Kashmir also reported that, the available nitrogen content ranged from 300 to 410 kg/ha. Akhtar (2005) while working in the apple orchard soils of Kashmir valley also reported the available nitrogen content in the range of 320 to 410 kg/ha.

Available phosphorus

Available phosphorus content of soils ranged between 16.21 kg ha⁻¹ (Rajpura) and 17.98 kg ha⁻¹ (Shiekhar) with an average value of 17.26 kg ha⁻¹. The available phosphorus content of Shiekhar soil was significantly more than soils of other villages except Shadimarg and Pinglin which were statistically at par. Khan *et al.*, (2013) reported that the available phosphorus was in the range of 10 to 24 kg/ha. The high available phosphorus may be due to application of organic manures like

FYM, vermicompost and biofertilizer. These findings are supported by Kumar *et al.*, (2009).

Available potassium

Available potassium content of soils ranged between 180.13 kg ha⁻¹ (Sunsomil) and 189.12 kg ha⁻¹ (Pinglin) with a mean value of 186.56 kg ha⁻¹. This is supported by the findings of Aon and Colaneri (2001) who also observed the potassium in range of 178.67 to 197.12kg/ha in the apple orchard soils of Himachal Pradesh.

Correlation studies

Table 6 revealed that correlation of AM spore population with organic C (r=0.887**), available N (r=0.815*), available K (r=0.614*) and root colonization (r=0.512*) were found to be positive and significant in District. However positive, but non-significant correlation was observed with respect to available P. The results are in conformity with those of Lipinski *et al.*, (2003) who also reported a significant positive correlation between soil organic carbon and AM spore population. The positive and significant correlation between AM spores and available nitrogen (r=0.815*) was found which might be due to the fact that nitrogen and organic carbon are required by micro-organisms for their special

requirements and as a result high nitrogen and organic carbon in the soil increased infection and population of AM fungi. Similar results were reported by Venkatrao *et al.*, (1972).

Between available nutrients of representative samples of District Pulwama

Perusal of the data present in Table 7 reveals that the organic carbon content of soils of representative villages was found to be positively and significantly correlated with available nitrogen ($r=0.931^{**}$), available sulphur ($r=0.923^{**}$) and available potassium ($r=0.784^*$) but correlation was positive and non-significant with available phosphorus.

The available nitrogen content of soils was positively and significantly correlated with organic carbon and available S while as positive and non-significant correlation was observed with available P and K. The available phosphorus content of soils was positive and non-significantly correlated with Organic C, available N, S and K contents. The available sulphur from soils was found to be positively and significantly correlated with organic carbon ($r=0.923^{**}$), available N ($r=0.803^{**}$) while as positive and non-significant correlation was recorded with Available P and K. The available potassium was found to be positively and non-significantly correlated with available N, P and S from the soils. However, the correlation with organic carbon ($r=0.784^*$) was positive and significant.

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