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Impact of Different Rice Farming Systems on Diversity of Arbuscular Mycorrhizal Fungi (AMF) in Selected Agroclimatic Zones of Andhra Pradesh

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ABSTRACT

The impact of different rice farming systems on the abundance and diversity of arbuscular mycorrhizal fungi (AMF) was investigated at selected four agro climatic zones of Andhra Pradesh *viz*., North Coastal Zone, Godavari Zone, Krishna Zone and Southern Zone. There was significant difference in AMF root colonization in different land use types during different cropping stages and also there was a significant difference in AMF root infection ratings between different farming systems, where it was higher in Natural farming. The AMF spore density was significantly higher in Godavari Zone compared to all other agro climatic zones. Seventy one AMF spore types obtained from different rice farming systems of selected 4 agro climatic zones of Andhra Pradesh during 3 cropping stages of rice were characterized based on spore morphology and they belong to 23 AMF species. Eleven AMF species from North Coastal Zone, 10 AMF species from Godavari Zone, 12 AMF species from Krishna Zone and 14 AMF species from Southern Zone were noticed. Regarding the frequency of distribution, *Glomus fasciculatum* was distributed more frequently in intensive farming system. While, in natural farming system *Acaulospora morrowae* was more frequently distributed. In organic farming system *Acaulospora lacunosa* was more frequently distributed. The genus *Glomus* was distributed more frequently than that in the genus *Acaulospora*. Shannon-Wiener diversity index of AM fungi was determined and it was more in organic farming system than other farming systems. Regarding agro climatic zones, AMF diversity was more in Krishna zone than other 3 zones. AMF diversity was more during grand growth stage compared to initial and harvesting stages. The available potassium and acid phosphatase had a positive influence on spore density and root colonization, while the available nitrogen, available phosphorus, alkaline phosphatase and bulk density had a negative influence on spore density and root colonization.

Keywords: *Agro climatic zones, Arbuscular mycorrhizal fungi (AMF), Farming systems, Per cent root colonization, Rice crop, Spore density, Spore types.*

Arbuscular mycorrhizal fungi (AMF) are ubiquitous root-symbiotic fungi in the phylum Glomeromycota formerly Glomales within the Zygomycota (Schussler *et al*. 2001). They form mutualistic associations with roots of the majority of higher plants, including crop plants. AM fungi exist in two different phases, inside the root and in the soil. The extraradical mycelium forms spores, explores soil and new areas for colonization and absorbs nutrients (Tommerup and Sivasithamparam, 1990).

In the recent system of classification, all the AM fungal species are placed in four orders *i.e.* Archaeosporales, Diversisporales, Glomerales and Paraglomerales which comprise 13 families and 19 genera that belong to class Glomeromycetes of the phylum Glomeromycota (Sieverding and Oehl, 2006; Oehl *et al*., 2008; and Palenzuela *et al*., 2008). Blaszkowski *et al.* (2015) presented a few important advances in the *Glomero myoto.*

AMF form symbiotic relationships with most terrestrial plants, including many crop plants. In exchange for the plant-assimilated carbon, the fungal partner benefits the host plant by facilitating plant access to mineral nutrients and water and by improving pathogen resistance (Smith and Read, 2008). In addition to these direct effects, the AMF community composition and diversity may also influence plant productivity and community structure (Van *et al.,* 2008).

In majority of the countries, rice is grown under waterlogged conditions. Recently, the occurrence of AMF in wetland ecosystems has received an increased attention (Wang *et al*., 2010, Baar *et al.,* 2011 and Moller *et al.,* 2013). Evidence is accumulating that AMF are not only present but also ubiquitous in some wetland ecosystems (Wilde *et al.,* 2009 and Wang *et al.,* 2011). The AMF communities of some wetland habitats have been investigated (Nielsen *et al.,* 200, Wilde *et al.,* 2009, Wang *et al.,* 2011, 4 Kohout *et al*., 2012), Moller *et al.,* 2013 and but whether AMF occur in paddy fields under waterlogged conditions is still under debate (Vallino *et al.,* 2014). Some studies found that AMF are able to survive in waterlogged paddy wetlands (Watanarojanaporn *et al.,* 2013), but there are also reports showing that AMF are absent or rare due to the anoxic conditions that often prevail in waterlogged systems (Lumini *et al*., 2011 and Vallino *et al.,* 2014).

Table 1. Bench mark points in selected agro climatic zones of Andhra Pradesh

S. No.	Agro Climatic Zone	Bench mark Location
1	North Coastal Zone	18° 51' 16" - 84° 58' 41". Palasa Puram, Srikakulam District.
2	Godavari Zone	17° 5' 33" - 82° 18' 47". Uppada-Pithapuram Road, Kottapalli, East Godavari District.
3	Krishna Zone	15° 56' 48" - 80° 37' 19". Allur-Khajipalem Road, Alakapuram, Guntur District.
4	Southern Zone	14° 26' 23" - 80° 8' 0" Vilukanipalli, Nellore District.

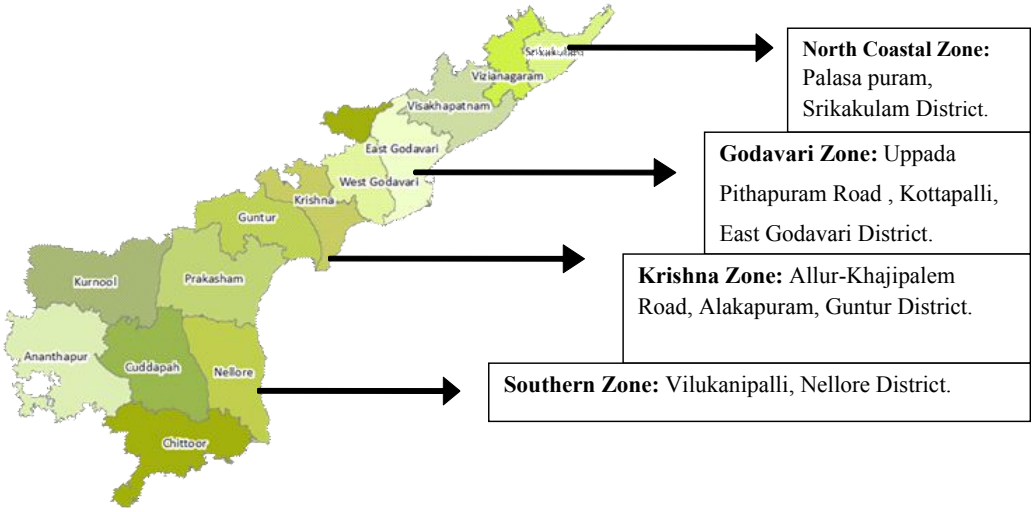

Fig 1. Locations of study sites in selected agro climatic zones of Andhra Pradesh

MATERIAL AND METHODS

Study Area

This benchmark areas situated in the North Coastal Zone, Godavari Zone, Krishna Zone and Southern Zone are presented in Table 1and Fig 1.

Soil sampling

Soil samples were collected from selected bench marks of four agro climatic zones (North coastal, Godavari, Krishna and Southern Zones) of Andhra Pradesh with respective farming systems (intensive, natural and organic). Soil samples were collected from 15-20 cm depth of the soil. Root bits were also collected along with soil samples during grand growth stage and before harvesting of the rice crop.

Root bits sample collection

Rice plant roots were collected from fields during grand growth and harvesting stage during *kharif,* 2017. The roots were washed with tap water to remove sand and soil. The roots were then cut into 1 cm bits. The root bits were preserved in a FAA (Formalin-Acetic acid-Acetone) till they are subjected for staining process.

Soil physico chemical properties

The pH was determined by using pH meter (Jackson, 1973). The EC was determined by using Conductivity bridge (Jackson, 1973). The available nitrogen in the soil samples was analyzed by semi micro-Kjeldhal method (Jackson, 1973). The available phosphorus content of the soil samples was determined by Olsen's method described by Olsen *et al*. (1954). The available potassium content of the soil samples was determined by Jackson (1973). The organic carbon content of the soil samples was estimated by Walkley and Black's wet oxidation method as outlined by Walkley and Black's (1934). The bulk density was determined following standard procedures (Anderson and Ingram, 1989). The acid and alkaline phosphatase activities were estimated as per Eivazi and Tabatabai (1977). The results of the soil analysis of different sites are given in the Table 2.

Assessment of the mycorrhizal parameters

The following mycorrhizal structures were determined: root colonization was determined by

gridline intersection method (Giovannetti and Mosse, 1980) by staining the root bits with 0.05% trypan blue. Spore density was determined by extracting spores from soil by wet sieving and decantation method (Gerdemann and Nicolson, 1963).

Identification of the diversity of AM fungi:

Spores extracted directly from soil were used for identification. Morphologically similar spores were picked and population of each spore type was enumerated. Spores of each type were brought into pot culture by funnel technique after surface sterilization of the spores (Nicolson, 1967). Surface sterilization of isolated spores was done with an aqueous solution containing 200-ppm streptomycin sulphate and 2% chloramine T. The spores were mounted on a glass slide in lacto- glycerol. They were later identified based on the spore morphology, with the help of "Manual for identification of VA mycorrhizal fungi" by Schenck and Perez (1990) and the INVAM website by Joe Morton.

Statistical analysis

The data collected from the field experiments were subjected to statistical analysis. Arcsin transformations were done as per the procedure given by Snedecor and Cochran (1968) wherever necessary. Three-way analysis of variance was used to analyse the data by CRD analysis and the Co-efficient of correlation was also worked out by the procedure outlined by Sundararaj *et al.* (1972) and by following the statistical package Excel and SYSTAT version 10.2. The treatment means were separated by the Dunkan's Multiple Range Test and by probability matrix. The Shanon-Weiner diversity index between different agro climatic zones was caluculated as out lined by Krebs (1989).

RESULTS AND DISCUSSION

Mycorrhizal root colonization:

AMF root colonization was studied in three different farming systems *viz*. Intensive farming system, natural farming system and organic farming system in selected four agro climatic zones during different cropping stages. Significant differences in root colonization between different farming systems, agro climatic zones and cropping stages were observed. AMF root colonization was significantly more in natural farming system compared to other rice farming systems. It was significantly more in Krishna Zone as compared to other three agro climatic zones. Regarding different cropping stages, root colonization was more in grand growth stage compared to harvesting stage.

AMF Spore density

The AM fungal spore density differed significantly between different farming systems, agro climatic zones and cropping stages, AM fungal spore density was more in natural farming system as compared to other farming systems and with respect to agro climatic zones, it was more in Godavari Zone compared to other zones. Spore density was more during harvesting stage than other stages.

Effect of soil properties on AM Fungi:

The influence of soil physical, chemical and bio chemical properties on different mycorrhizal parameters was determined. Among the physical, chemical and biochemical properties of the soil in the study site, available potassium and acid phosphatase positively correlated with AMF spore density and root colonization while the available nitrogen, available phosphorus, alkaline phosphatase and bulk density were negatively correlated with AMF spore density and root colonization (Table 2).

Species abundance and diversity of AM fungi from different farming systems in four selected agro climatic zones during different cropping stages:

There were 71 AMF spore types isolated from three farming systems in four selected agro climatic zones during three cropping stages of rice cultivation. These 71 AMF spore types were characterized up to species level based on spore morphology. The spore types were belonging to 23 AMF species.

In intensive farming system, there were 6 AMF species present in North Coastal Zone, 4 in Godavari Zone, 6 in Krishna Zone and 7 in Southern Zone. In natural farming system, there were 9 AMF species present in North Coastal Zone, 5 in Godavari Zone, 5 in Krishna Zone and 4 in Southern Zone. In organic farming system, there were 5 AMF species present in North Coastal Zone, 5 in Godavari Zone, 8 in Krishna Zone and 5 in Southern Zone.

Table 2. Correlation matrix between soil properties and AM fungi in different farming systems

Soil properties	Root colonization (%)	Spore density (100 g^{-1} of soil)
Available N	-0.066	0.189
Available P	-0.698	0.06
Available K	0.581	0.517
Alkaline phosphatase	-0.08	-0.96
Acid phosphatase	0.823	0.678
Bulk density	-0.421	0.781

Table 3. Abundance of different species of AM fungi from different farming systems in selected agroclimatic zones of Andhra Pradesh

AM fungal species	AMF spore abundance (No. of spores $100g^{-1}$ soil)	Spore abundance (%)
NORTH COASTAL ZONE:-		
Intensive farming system:		
Acaulospora lacunosa	357	32.3
A. morrowae	103	9.32
Glomus clarus	11	0.99
G. fasciculatum	371	33.57
G. mosseae	137	12.4
G. multicaulis	126	11.4
Natural farming system:		
Acaulospora lacunosa	68	6.63
A. morrowae	72	7.02
Glomus clarus	144	14.04
G. diaphanum	89	8.68
G. dimorphicum	9	0.87
G. fasciculatum	303	29.56
Glomus hoi	252	24.58
G. intraradices	140	13.65
G. monosporum	8	0.78
Organic farming system:		
Acaulospora lacunosa	373	37.15
A. morrowae	285	28.38
Glomus fasciculatum	165	16.43
G. intraradices	17	1.69
G. monosporum	164	16.33
GODAVARI ZONE:-		
Intensive farming system:		
Acaulospora appendicula	518	38.34
A.bireticulata	423	31.31
Glomus maculosum	201	14.87
A. morrowae	412	19.59
Glomus hoi	502	23.87
G. mosseae	899	42.74
Organic farming system:		
Acaulospora lacunosa	148	10.73
Glomus hoi	288	20.88
G. lacteum	387	28.06
G. mosseae	276	20.01
G. multicaulis	280	20.3
KRISHNA ZONE:-		
Intensive farming system:		
Acaulospora	139	11.7
Glomus fasciculatum	210	17.67
G. geosporum	235	19.78
G. heterosporum	13	1.09
G. hoi	352	29.62
G. monosporum	239	20.11
Natural farming system:		
Acaulospora lacunosa	167	14.91
A. morrowae	214	19.1
Glomus fasciculatum	444	39.64
G. intraradices	295	26.33
Organic farming system:		
Acaulospora lacunosa	127	10.13
A.morrowae	153	12.21
Glomus fasciculatum	132	10.53
G. geosporum	121	9.65
G. heterosporum	92	7.34
G. hoi	252	20.11
G. intraradices	70	5.58
G. macrocarpum	21	1.67
G. mosseae	265	21.14
G. versiforme	20	1.59
SOUTHERN ZONE:-		
Intensive farming system:		
Acaulospora lacunosa	122	10.08
A. morrowae	311	25.7
A. scrobiculata	123	10.16
Glomus fasciculatum	138	11.4
G. hoi	295	24.38
G. macrocarpum	134	11.07
G. phansihalos	87	7.19
Natural farming system:		
Natural farming system:		
Acaulospora	656	43.79
Glomus intraradices	515	34.37
G. monosporum	307	20.49
G. versiforme	20	1.33
Organic farming system:		
Acaulospora lacunosa	120	9.98
A. morrowae	285	23.71
Glomus etunicatum	149	12.39
G. fasciculatum	149	12.39
G. heterosporum	140	11.64
G. intraradices	107	8.9
G. lacteum	237	19.71
G. reticulatam	15	1.24

Table 4. Frequency distribution of different species of AM fungi over different farming systems of Andhra Pradesh

AM fungal species	Frequency of distribution (out of 8 sampling points)			Distribution (%)		
	Intensive farming system	Natural farming system	Organic farming system	Intensive farming system	Natural farming system	Organic farming system
Acaulospora appendicula	1	ND	ND	12.5	ND	ND
A. bireticulata	3	2	ND	37.5	25	ND
A. lacunosa	3	3	7	37.5	37.5	87.5
A. morrowae	3	6	5	37.5	75	62.5
A.scrobiculata	1	ND	ND	12.5	ND	ND
Glomus clarus	2	1	ND	25	12.5	ND
G. diaphanum	ND	1	ND	ND	12.5	ND
G.dimorphicum	ND	1	ND	ND	12.5	ND
G. etunicatum	ND	ND	ND	ND	ND	ND
G.fasciculatum	4	4	4	50	50	50
G. geosporum	1	ND	ND	12.5	ND	ND
G. heterosporum	2	ND	1	25	ND	12.5
G. hoi	2	3	1	25	37.5	12.5
G. intraradices	ND	5	4	ND	62.5	50
G. lacteum	ND	ND	3	ND	ND	37.5
G.macrocarpum	1	ND	ND	12.5	ND	ND
G. maculosum	1	ND	ND	12.5	ND	ND
G. monosporum	ND	3	2	ND	37.5	25
G. mosseae	1	3	3	12.5	37.5	37.5
G. multicaulis	1	ND	1	12.5	ND	12.5
G. pansihalos	1	ND	ND	12.5	ND	ND
G. reticulatum	ND	ND	2	ND	ND	25
G. versiforme	ND	1	ND	ND	12.5	ND

The spore abundance was more in Godavari Zone than all the different agro climatic zones. In North Coastal Zone, *Glomus fasciculatum* was more abundant in intensive and natural farming system and *A. lacunosa* was abundant in organic farming system. In Godavari Zone, *A. apendiculata* was more abundant in intensive, *G. hoi* was more abundant in natural farming system and *G. lacteum* was abundant in organic farming system. In Krishna Zone *G. hoi* was more abundant in intensive, *G. fasciculatum* was more abundant in natural farming system and *G. mosseae* was abundant in organic farming system. In Southern zone, *A. morrowae* was more abundant in all the farming systems. (Table 3)

The frequency distribution of AM fungi in different farming systems in different agro climatic zones revealed that, *G. fasciculatum* was distributed more frequently in intensive farming system. While, in natural farming system *A. morrowae* was more frequently distributed. In organic farming system *A. lacunosa* was more frequently distributed. (Table 4).

Regarding the distribution of different genera of AM fungi in three rice farming systems of four agro climatic zones of Andhra Pradesh during three cropping stages, *Glomus* was distributed in all agro climatic zones compared to *Acaulospora*.

Diversity of AM Fungi from different farming systems in four selected agro climatic zones during different cropping stages:

Shannon-Wiener diversity index of AM fungi differed significantly among different farming systems, agro climatic zones and cropping stages Shannon-Wiener diversity index was more in organic farming system as compared to other farming systems and with respect to agro climatic zones, it was more in Krishna compared to other zones. Shannon-Wiener diversity index was more during initial stage than other stages.

The present investigation in the different farming systems of selected agroclimatic zones of Andhra Pradesh has shown that more intensive farming system has a negative impact on AMF spore density

and per cent root colonization and thus in principle, some earlier studies on similar subjects *e.g*. Oehl *et al*. (2003) confirmed a decrease in AMF species richness with increasing land use intensity in Central Europe.

AMF spore density

There was a significant difference between agro climatic zones, farming systems and crop stages of the rice. A comparison of the AMF spore density revealed, that it was significantly higher in Godavari Zone compared to other agro climatic zones. Further, even in different farming systems the AMF spore density was significantly higher in natural farming system compared to other farming systems. Among the different cropping stages, grand growth stage had significantly higher AMF spore density compared to other cropping stages.

Similar results were obtained in the earlier studies where Lahrmann *et al*. (2013) noticed the increas-ed root colonization and AMF spore production at high light intensity. Kowalczyk and Blaszkowski (2011) noticed that AMF spores from the genus *Glomus* were predominate in Poland's cultivated soils, which might be related to pH, fertilization and better structure of the soils. Lekberg *et al*. (2008) noticed that geographical location and topographic differentiation of cultivated soils as well as the variability of climatic factors affect the population of AMF in the soils and their symbiotic activity. Optimum temperature for the activity of AMF is similar to the optimum range needed for plant vegetation. They have observed that the temperature optimum was varied for different developmental stages of AMF (germination, infectiveness of hyphae, formation of arbuscules and vesicules, sporulation). Gosling *et al*. (2006) noticed that in agricultural soils, considerable differentiation in the composition of AMF spore population existed. This is connected with the methods of cultivation and additional agro chemical treatments. Brundrett *et al.* (1996) found that AMF spores had a significant correlation with soil texture, *i.e*. positive for clay content and negative for sand content of the natural soil habitat in Australia.

Ilag *et al*. (1987) have noticed that AMF populations were depleted by flooded rice. Field sampling three sites in Philippines revealed that the infective AMF population was very low after wet season rice, but it increased again during the maturity of dry season rice.

Influence of soil properties

Soil physical, chemical and biochemical properties like available potassium and acid phosphatase had a positive influence on spore density and root colonization, while the available nitrogen, available phosphorus, alkaline phosphatase and bulk density had a negative influence on spore density and root colonization.

Similar findings were reported by earlier workers where Koide (1991) reported that high P content (>9ppm) reduced mycorrhizal colonization and spore production. Further, Lakshmipathy *et al.* (2003) while studying AM colonization in medicinal plants of Western Ghats also obtained similar results. A significant positive correlation existed between mycorrhizal activity and acid phosphatase activity. Mycorrhizal colonization is known to alter the inherent phosphorus supply by increasing the phosphatase activity in the rhizosphere (Azcon *et al.,* 1982). The present study also upholds the observations made by earlier workers (Lakshmipathy *et al.,* 2003; Sumana and Bagyaraj, 1996).

AMF species distribution:

This kind of variation in AM fungal species composition over different agroclimatic zones and farming systems could be attributed to adoption of specific AMF species to a particular climatic condition, management practices and soil moisture regimes. Lovelock (2003) also observed that the relative abundance of spores of *Acaulospora* was lower than that of *Glomus* during wet season and found that *Glomus* produced relatively more spores at highest seasonal rainfall. Further, he also suggested that profuse rooting favoured the sporulation of AMF species.

Schenck and Kinloch (1980) also noticed incidence of AM fungal species over different periods of time over the years.

Difficulties in predicting levels of indigenous AMF populations in different soils arise from the large number of factors that can affect their contribution, activity and survival. These include soil fertility, soil moisture, pH, plant susceptibility, light intensity, altitude, soil organic matter, depth and soil disturbance physical movement by water, earthworms and soil microfauna. The wide range of AM fungi in many natural habitats suggest a degree of ecological equivalence between species (Hayman, 1978; Molina *et al.* 1978). Likewise, similar agricultural soils growing the same crop may contain different species. Furthermore, chemicals added to agricultural soils can change the species composition as well as the total size of the mycorrhizal population and the indigenous mycorrhizal populations of natural soils are often very sensitive to soil amendments. Further, it is quite evident that AMF generic distribution pattern varies with the soil type, vegetation, season and change in land use types. Muthukumar and Udaiyan(2000) and Mohan, (2003) have reported preponderance of species of *Glomus* and *Acaulospora* in Indian soils under tropical

conditions. Mohan (2003) recorded 47 species of *Glomus* and 16 species of *Acaulospora* from soils under forest plantations in Western Ghats. Probably, soil pH may play a crucial role in the distribution of these fungi. Porter *et al*. (1987) have reported that *Glomus spp*. was of rare occurrence in Western Australia due to high pH. This suggests, that the wide distribution of *Glomus* and *Acaulospora* could be pH dependent and this could be one of the reasons why these genera are predominant in the present study where the pH of the soils are acidic.

AMF species spore abundance in different farming systems

In the present study, it was observed that there were variations in AM fungal spore density in different farming systems and different agro climatic zones. It was found that the spore density were significantly more in Godavari Zone compared to other zones and Natural farming system have more AM fungal spore density. Different climatic nature, farming practices, topography favoured better mycorrhization, in turn resulted in more sporulation of AM fungi.

De Souza *et al.* (2013) investigated the seasonal dynamics of arbuscular mycorrhizal (AM) fungal community composition in pre monsoon and post monsoon seasons. Variation in spore abundance of the AMF species in different farming systems, agroclimatic zones and cropping stages might be due to variation in soil type, pH, EC, age of the crop, cultivation practices *etc*. Lumini (2010) and Borriello (2012) have noticed that AMF diversity, effectiveness and abundance declined in agroecosystems subjected to high input practices. Verbruggen *et al.* (2010) noticed most human activities have an arguable impact on the physical and biological aspects of soil.

Diversity of AM fungi in different farming systems

Variation in diversity of AM fungi in different land use types and during different seasons was noticed even in earlier studies. Mycorrhizal fungi are likely to be affected by plant community composition, cultivation practices and soil properties (Janos, 1980; Kormanik *et al.,* 1980). The lower spore production is probably associated with the ability of the fungus to spread by hyphal growth from root to root and thus save the energy needed for sporulation (Janos, 1975).

Oehl *et al.* (2003) in their study also recorded highest mycorrhizal diversity index in grasslands compared to moderate and low input arable lands and intensive continuous maize mono-cropping. Carpenter *et al*. (2001) in their study on spore density and diversity of AM fungi in different land uses found that diversity of AM fungi changed due to change in farming systems. Further, Acacia plantations, which have been established in abandoned soils, have also shown a good diversity which is a similar observation made by Oehl *et al.* (2003). However, Rashi *et al.* (1997) did not find any significant difference in AMF diversity due to change in land use types. Lakshmipathy *et al*. (2012) observed variation in AM fungal diversity across different land use types in Western Ghats of Karnataka.

CONCLUSION

This study showed that the changes observed in soil physico-chemical and biochemical properties in different farming systems, agro climatic zones and cropping stages during rice cultivation might be responsible for variation in AMF activity and diversity. Further, the variations in soil properties in different farming systems, agroclimatic zones and cropping stages also had an influence on soil micro flora other than AMF. This might varied the organic C and other nutrients. Hence, the variations were noticed in AMF activity in different farming systems, agro climatic zones and cropping stages of rice. Favourable conditions which existed in Organic and natural rice farming systems enhanced the AMF spore density, percent root colonization and diversity.

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