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Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Spore population, colonization, species diversity and factors influencing the association of arbuscular mycorrhizal fungi with litchi trees in India

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Publication Info

Paper received:
11 May 2014

Revised received:
30 May 2015

Re-revised received:
01 July 2015

Accepted:
08 August 2015

Abstract

Abundance and diversity of arbuscular mycorrhizal fungi (AMF) in association with litchi (*Litchi chinensis* Sonn.) trees were studied during 2012-2013, where orchard soil had high pH (7.42-9.53) and salinity (0.07- 0.39 dSm^{-1}). A total of 105 rhizospheric soil and root samples were collected considering variables like location, age of tree, cultivar and production management. Results showed that spore count was in the range of 1-22 g^{-1} soil. All the examined root segments had colonization of AMF, which ranged between 3.3 to 90.0%. AMF community comprised of *Glomus mosseae, G. intaradices, G. constricta, G. coronatum, G. fasciculatum, G. albidum, G. hoi, G. multicauli, Acaulospora scrobiculata, A. laevis, Rhizophagus litchi* and *Entrophosphora infrequens*. Higher spore density and AMF colonization were observed at medium level (13-28 kg ha^{-1}) of available phosphorus that decreased ('r' = -0.21 for spore density, -0.48 for root colonization) with increasing soil phosphorus. While nitrogen did not influence the AMF association, a weak negative linear relationship with AMF colonization ('r'= -0.30) was apparent in the medium level (112-200 kg ha^{-1}) of potash. Micronutrients (Zn, Fe, Cu, Mn and B) did not affect spore density (zero or a very weak linear correlation) but influenced root colonization ('r'= -0.53 to -0.44), the effect being more prominent above critical limits. Nutritionally sufficient, irrigated litchi orchards had greater spore count (46% samples having 5-22 spores g^{-1} soil) and colonization (>50% in 37.4% roots examined) than nutrient deficient, non-irrigated orchards, indicating essentiality of a threshold nutrients and moisture regime for the association. AMF symbiosis was influenced by cultivar (greater in 'China'), but tree age was not correlated to mycorrhizal association. A consortium of native species coupled with the understanding of nutrient effects on AMF would be useful for field application in litchi.

Key words

Arbuscular mycorrhiza, *Litchi chinensis*, Root colonization, Soil nutrients, Spore density

Introduction

Arbuscular mycorrhizal fungi (AMF) are widespread symbiotic partners with majority of land plants (Wang and Qiu, 2006). AMF obtain all their carbon from host plants primarily in return for improved access to phosphorus and, to a smaller extent, nitrogen (Fitter 2006; Guether *et al.,* 2009; Kiers *et al.,* 2011). Janos *et al.* (2001) reported that inoculation with AMF enhanced growth of litchi trees after propagation by air-layering. Sharma *et al.* (2009) noticed qualitative and quantitative differences in AMF species with different cultivation types in litchi growing areas of North-Western Himalayan Region (NWHR) of India. A marked reduction was noted in the AMF where chemicals were used for weed control and intensive farming system was used on the orchard floor. A good colonization of litchi trees in Western Himalayas has been reported (Aradhana *et al.,* 2013). These reports clearly show the importance of AMF association with litchi, however, knowledge about the factors that determine AMF community structure and symbiotic

functioning is limited. Development oa AMF may depend on the edaphic conditions (He *et al.*, 2002; Morammad *et al.*, 2003) or climatic conditions (He *et al.*, 2002; Muthukumar and Udaiyan, 2002). Recent findings suggest that adaptation of AMF to abiotic factors such as temperature and nutrient availability can strongly influence the effect of AMF symbiosis on plant growth (Johnson, 2010; Antunes *et al.*, 2011). However, no such studies have been done in litchi orchard ecosystem. An understanding of soil nutrient effect on AMF symbiosis besides biotic factors will be helpful to harness the AMF association for enhanced productivity and quality of litchi. In litchi growing areas of Bihar, which is the 'litchi hub' of the country, orchard soils are saline having high pH where phosphorus is fixed by calcium making it unavailable to trees. The growth of seedlings and trees may be improved by use of native mycorrhizal inoculants. Therefore, the aim of the present study was to examine the abundance of AMF, extent of root colonization and species diversity of AMF and further, to understand the soil nutrient effect on AMF symbiosis in litchi besides cultural and biotic factors.

Materials and Methods

Site description : The study was carried out at the National Research Centre on Litchi, Mushahari, Muzaffarpur. The rhizosphere soil samples were collected from litchi orchards located in five districts *viz.*, Muzaffarpur, Vaishali, Samastipur, East Champaran and West Champaran of Bihar state of India. These regions come under the northwest alluvial plain zone and has a subtropical climate characterized by hot summer, wet monsoon and dry winter. The soil of the region is alluvial in nature.

Collection of rhizosphere soil and root samples : Samples were collected randomly from active root feeding zone that was about 1-2 feet inward from the apparent boundary of tree canopy on land. After removing top soil and grasses, rhizosphere soil along with fine root bits were collected from 0-20 cm depth in three replicates per tree, pooled and thoroughly mixed. Finally, about 500 g sample was retained and placed in individual plastic bags. Besides distant location, other variables considered for sampling were age of tree (10-50 yr), cultivar ('Shahi' or 'China') and production management (well managed vs. neglected or poorly managed). In laboratory, half of the collected samples were placed in refrigerator at 4 °C for enumeration of spores and root colonization studies, and the remaining half were air dried, ground and passed through 2 mm sieves for soil analysis and to establish successive pot cultures (trap cultures).

Extraction and estimation of AMF spores : AMF spores were extracted using wet sieving and centrifugation method (Brundrett *et al.* 1996). 10 g air dried soils was suspended in tap water and decanted through 4 stacked sieves of size 710, 500, 150, and 63 µm. Samples sieving collected from the sieves 500, 150, and 63 µm were taken with water and centrifuged at 3000 rpm (1409 x g). The pellets formed were resuspended in 1.17 M sucrose solution and centrifuged. Spores in sucrose supernatant were then filtered through grid pattern filter paper and washed with distilled water to spread spores evenly over the entire grid. They were counted under a stereoscopic microscope (40 x) before being separated based on colour and size. The number of spores was expressed as mean of three replicates.

Trap cultures : Trap cultures of the field-collected spores were used for taxonomic identification of AMF species. For this, maize (*Zea mays*) plants were grown in pots having 250 g dry field soil mixed with autoclaved sand (1:1, v/v). Seeds were surface-sterilized in 1% sodium hypochlorite for 1 min and then washed with distilled water before planting in pots.

Identification of AMF : Diagnostic slides with spores/ sporocarps were prepared by mounting onto a slide in Meltzer's reagent mixed with polyvinyl lactoglycerol (PVLG) (1:1, v/v). All the spores (including broken ones) were examined under fluorescent trinocular upright microscope. Taxonomic identification of spores up to species level was based on spore size, spore colour in Melzers' reagent, wall layers and hyphal attachments using identification manual (Schenck and Perez, 1990) and reference of International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (http://invam.caf. wvu.edu). Relative abundance of AMF was determined by the following formula: number of spores of a species or genus/total spores ×100. Frequency of occurrence (%) for each AMF species was calculated as a fraction of number of soil samples possessing spores of the species.

Assessment of root colonization by AMF : Root samples (2 g fresh weight) were separated from soil, washed in tap water and cut into 1 cm pieces. These were then placed in 10% KOH solution in beaker and autoclaved at 15 lbs pressure for about 15 min. The samples were then cooled and KOH was decanted. Thereafter, root samples were bleached with freshly prepared alkaline hydrogen peroxide (3 ml of 25% ammonia solution + 30 ml of 6% H_2O_2 v/v) for 20-30 min. After this alkaline hydrogen peroxide was decanted and roots were treated with 1N HCl for about 10 min followed by keeping it 0.05% Trypan blue in lactic acid-glycerol for staining overnight. After removing from stain, roots were placed in lactoglycerol destaining solution. These were then examined at 40x magnification and AMF root colonization were estimated by the gridline intersection method (Giovanetti and Mosse, 1980). Further, for assessing vesicular and arbuscular colonization, 30 randomly selected

root segments per sample were examined at 400-600x magnifications under fluorescent trinocular upright microscope by slide method and colonization percentage was calculated.

Analysis of soil parameters : Soil samples were analysed for pH and electrical conductivity in a ratio of 1:5 soil: water suspension. Organic carbon was estimated using 1 N potassium dichromate and back titrated with 0.5 N ferrous ammonium sulphate solution (Walkley and Black, 1934). Micro-Kjeldahl method was used to determine total N (Foster, 1995). Available phosphorus in soil was determined by extraction with 0.5 M sodium bicarbonate for 30 min (Olsen *et al*, 1954). AB-DTPA extractable P, K, Cu, Fe, Zn, B, S and Mn were analyzed following the method of Soltanpour and Schawab (1977). Quantification was done using atomic absorption spectrophotometer.

Statistical analysis : Data were analyzed using SAS® 9.2 statistical computing software. Spore counts were square root transformed before analysis of variance (ANOVA). Correlation analyses were performed to evaluate the relationship between spore numbers, percent root colonization, and various parameters influencing the association. Data were plotted on a scatter diagram.

Results and Discussion

A high level of spore density of AMF was recorded in rhizospheric soil of litchi, and a statistically significant difference in mean spore count per gram soil (LSD 1.77 ± 0.63, $P = 0.05$) was observed among the orchards. The spore count of AMF ranged from 1 to 22 per gram soil. Out of 105 samples, the average number of spores per gram soil was 1.0-5.0 in 58 samples, 5.1-10.0 in 35 samples, 10.1-15.0 in 9 samples, 15.1-20.0 in 2 samples, and one sample had more than 20 spores (Fig. 1). The abundance of spores in rhizosphere indicates potential of AMF in litchi. Average moisture level in soil samples was about 17-20% that was favourable for high spore density. Hindumathi and Reddy (2011) reported similar findings in rhizosphere of brinjal. Though spore numbers may poorly reflect the colonization potential of soil and are not always related to rate and extent of mycorrhizal formation, the availability of AMF spores may restrict the extent and time of colonization of litchi roots.

All the root segments observed were colonized by AMF and colonization ranged from 3.3 to 90.0% (Fig. 2). Distribution of root samples under different percent colonization category is given in Table 1. It is evident from data that majority of the samples had root colonization below 60%. Roots having presence of arbuscular, vesicular and both type of colonization were up to 50.0, 66.6 and 46.6%, respectively. Distribution of root samples of litchi having these three types of colonization is presented in Fig. 3. Majority of root samples had vesicular colonization <20%, arbuscular colonization <20%, and both arbuscular plus vesicular colonization <10%. Further, under zero arbuscular, vesicular, both vesicular plus arbuscular colonization category 13.3, 1.9 and 24.8 % root samples were present.

The study confirmed widespread occurrence of AMF in association with litchi trees at different locations though

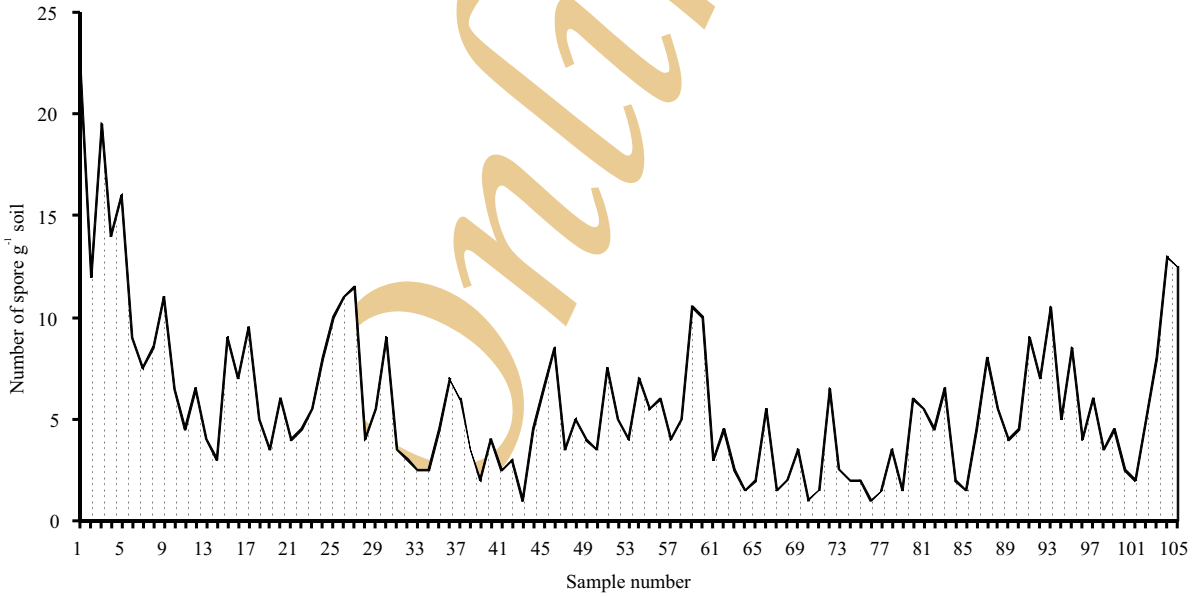

Fig. 1 : Average spore count of AMF in rhizospheric soil of litchi during 2012-2013 from different locations of Bihar, India

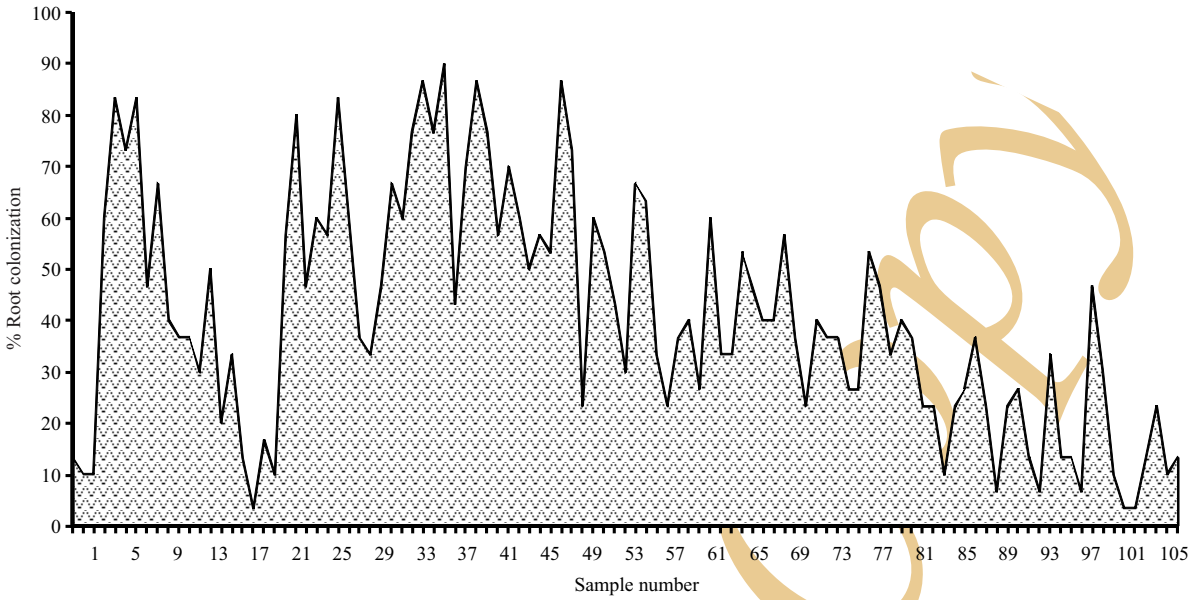

Fig. 2 : Colonization of roots of litchi by arbuscular mycorrhizal fungi during 2012-2013 from different locations of Bihar, India

Table 1: Distribution of root samples of litchi under different percent colonization category

Samples	Percent colonization									
	Zero	**1-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**81-90**
No. of samples	0	12	9	17	22	10	16	6	6	7
% samples	0	11.4	8.6	16.2	21	9.5	15.2	5.7	5.7	6.7

Table 2: Assay value of physico-chemical parameters in soil samples of litchi orchards

Soil variable	Assay value (range) in samples	Critical limit for litchi*	Number of samples in various level**(Total no. samples =105)		
			Low	**Medium**	**High**
% Organic carbon	0.02-1.05	NA	53 (<0.5)	43 (0.5-0.75)	9 (>0.75)
% Organic matter	0.21-1.81	NA	105 (<2)	0 (2-4)	0 (>4)
% Nitrogen	0.01-0.09	NA	7 (<0.03)	89 (0.03-0.06)	9 (>0.06)
N(kg/ha)	232-2027	250	1 (<280)	8 (280-560)	96 (>560)
Available $P_2 0_5$ (kg/ha)	4.57-137.1	25	4 (<10)	58 (11-25)	43 (>25)
Available K_2O (kg/ha)	69-293	125	13 (<112)	(112-280)	1 (>280)

*Source: Ray, P.K. (2004) ** Value in parenthesis is critical range; Source: Koley, A.K. (1933); NA=Not available

there was considerable variation in percent root colonization and number of spores in rhizospheric soil. Data revealed that spore density and root colonization was not correlated with each other in a 'cause and effect' relationship. Lack of correlation between number of spores and percent root colonization might be due to several reasons such as some species of AMF need longer time to germinate and some others seems not to have the capability of germination, spores might be lost during periods of mycorrhizal formation, or as a result of predation by soil organisms, as well as due to the impact of adverse soil conditions during inactivity period (Ghorbani *et al*., 2012). Further, there are fungi that sporulate more while others sporulate less (perhaps never) and others sporulate only during certain period of the year (Moreira *et al*., 2006).

Altogether, thirteen taxa of AMF were isolated and identified. Among these, 8 species belonged to genus *Glomus*, 2 species to *Acaulospora* and 1 species each to *Rhizophagus, Entrophosphora* and *Scutellospora*. *Glomus*

was observed to be predominant followed by *Acaulospora* in the rhizosphere soil of litchi. Different AMF species identified were *G. mosseae, G. intaradices, G. constricta, G. coronatum, G. fasciculatum, G. albidum, G. hoi, G. multicauli, Acaulospora scrobiculata, A. laevis, Rhizophagus litchi* and *Entrophosphora infrequens.* Among these, *G. mosseae, G. intaradices* and *G. fasciculatum* were identified as dominant species based on isolation frequency (IF) and relative abundance (RA), as these species exhibited >50% IF and >30% RA. Multiple species of AMF can colonize roots of individual plant species, but factors that determine selection of a particular AMF species in plant root are largely unknown. Wide occurrence of genus *Glomus* in the present study, as well as, in reports of several workers (Zhao *et al.*, 2003; Gai *et al.*, 2009) suggested that genus *Glomus* has a wide ecological amplitude that is responsible for its adaptability and survival in different habitats. Difference in AMF species in various samples can be

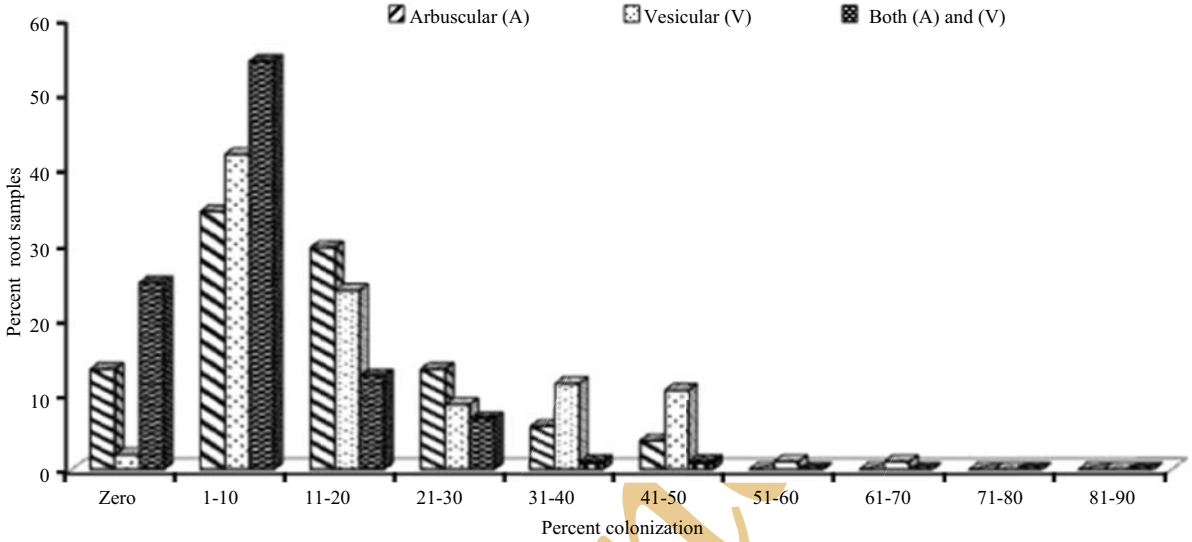

Fig. 3 : Distribution of root samples having presence of arbuscular, vesicular and both type of colonization

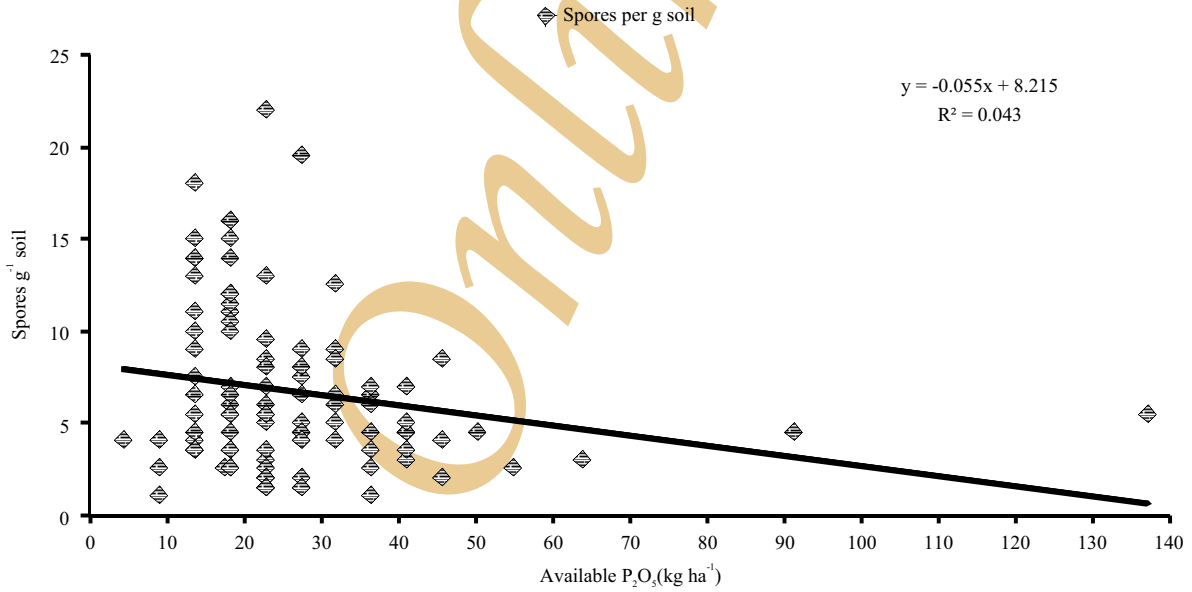

Fig. 4 : Correlation between available phosphorus and spore density of AMF in rhizospheric soil of litchi

attributed to changes in soil chemical properties resulting from cultural practices such as ploughing, application of chemical fertilizers and weedicides. Menendez *et al.* (2001) found that *Glomus* was more resilient while *Entrophospora* was more sensitive to tillage. Antunes *et al.* (2012) found that long-term use of specific soil fertilization treatment not only affected the overall abundance and diversity of indigenous AMF in an agricultural field, but also altered the effect of AMF symbiosis on plant and fungal growth. The AMF community structure in litchi is first time described in the present study. The native isolates from litchi ecosystem, where soils are saline, will be useful as inoculants for field application in this region.

The results of soil analysis showed that pH and electrical conductivity of soil in litchi orchards were ranged from 7.42 to 9.53 and 0.07 to 0.39 dSm^{-1}, respectively. Soil pH in the range of 7-8 was reported to be optimal for mycorrhizal symbiosis (Khakpour and Khara, 2012). AMF behaviour is affected by soil pH and influence mycorrhizal establishment and growth of plant (Carrenho *et al.*, 2007). It may limit the availability of nutrients; influence the pattern of absorption of nutrient and their exchange in root zone and even distribution of AMF species. A weak positive correlation of pH with AMF root colonization ($r = 0.41$, $R^2 = 0.17$) was evident but no correlation with spore count was apparent. This result is in agreement with the observation of

Table 3 : Assay value of micronutrients in soil samples of litchi orchards

Nutrients	Assay value in samples (ppm)	Critical limit (ppm) for litchi*	Number of samples	
			<Critical limit	≥Critical limit
Zn	0.18-3.32	0.78	67	38
Fe	3.42-27.2	7.0	14	91
Cu	0.16-11.69	0.53	2	103
Mn	3.18-30.86	3.0	0	105
B	0.12-5.94	0.53	11	94
S	0.94-45.63	10.0	96	9

*Source: Ray, P.K. (2004)

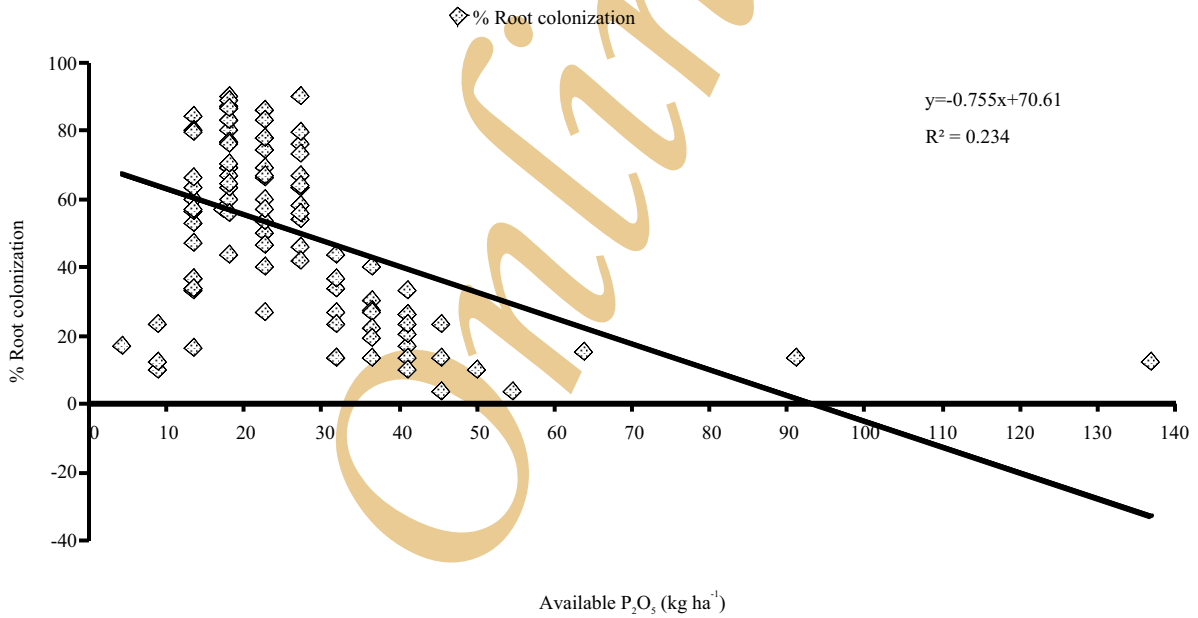

Fig. 5 : Correlation between available phosphorus and colonization of roots by AMF in litchi

Akond and Khan (2001) who reported a positive effect of pH on root colonization ($r = 0.56$, $p<0.01$) but not in spore density however, Khakpour and Khara (2012) reported that number of spores were negatively correlated with pH. Similarly, Rajeshkumar *et al*. (2015) reported that coconut palm cultivated in crop mixed system under rain-fed condition, soil pH had negative correlation with spore count and root colonization.

Soil salinity, in terms of electrical conductivity (EC), negatively affected spore density ($r = -0.34$, $R^2 = 0.11$) and root colonization ($r = -0.45$, $R^2 = 0.20$). Maximum spore density and root colonization were observed at 0.1 dSm^{-1} EC. Khakpour and Khara (2012) and Ghorbani *et al.* (2012) also observed that EC had a strong negative correlation with number of spores and percent root colonization. Many studies have evaluated the influence of organic matter on arbuscular mycorrhiza (Gaur and Adholeya, 2002) with varying results, indicating variable response on plants and fungi. In the present study, organic matter content showed weak correlation with spore density ($r= 0.17$, $R^2 = 0.03$) and root colonization by AMF ($r = -0.16$, $R^2 = 0.03$) which is in confirmation with the findings of Lingfei *et al.* (2005). In majority of the samples organic carbon was found to be low (<0.5% in 53 samples).

The data summarized in Table 2 shows that among major nutrients, majority of the samples showed high nitrogen (>560 kg ha^{-1} in 96 samples) but available phosphorus (11-25 kg ha^{-1} in 58 samples) and potash (112-280 kg ha^{-1} in 91 samples) was found in medium range. Available phosphorus was high (>25 P_2O_5 kg ha^{-1}) only in 43 samples. Analysis of data revealed that N content of soil did not have any influence on either spore density in rhizosphere or root colonization by AMF. This is in contrast to Sharma *et al.* (2009) and Ghorbani *et al.* (2012) who observed comparatively higher AMF spore population supported by higher available N in soil. In contrast to N, a medium level of available phosphorus (P_2O_5) (13-28 kg ha^{-1}) showed positive effect on spore density and root colonization by AMF. A negative linear correlation was apparent when data was plotted on scatter diagram, that was more strong with respect to root colonization ($r = -0.48$, $R^2 = 0.23$) than spore density ($r = -0.21$, $R^2 = 0.04$) (Fig. 4 and 5). This is in agreement with the study of Aliasgharzadeh *et al.* (2001). Coefficient of determination (R^2) values indicated that 23% of the variability in extent of root colonization could be explained by available phosphorus content, it was responsible for only 4% of the observed variability in spore density of AMF in rhizosphere. The P content of soil is the main factor that regulates establishment and efficiency of AMF symbiosis, which might either be due to direct effect of P on the external hyphal growth or indirect effect associated with the P status of plant (Ghorbani *et al.,* 2012). Nouri *et al.* (2014) showed that P and N can potentially exert negative regulation on AMF, whereas Mg^{2+}, Ca^{2+}, and Fe^{3+} ion had no effect. On the other hand starvation for several mineral nutrients, in particular for nitrate, reversed the inhibitory effect of P on AMF indicating that nutrient starvation triggers a dominant AMF-promoting signal that counteracts the effects of high P.

Potassium level (K_2O) in rhizosphere soils ranged from 69-293 $kgha^{-1}$. Maximum number of spores of AMF and root colonization was observed, where potassium in soil was found in medium range (112-200 kg ha^{-1}). When data was plotted on a scatter diagram, no apparent correlation was found with spore density ($r = -0.05$, $R^2 = 0.00$) but a weak negative linear correlation was observed with respect to root colonization ($r = -0.28$, $R^2 = 0.08$), which was prominent at higher potassium level in soil. Sreevani and Reddy (2004) and Motha *et al.* (2014) also found high potassium level in soil rhizosphere of tomato while brinjal had least number of AMF spores. Soil K has been reported to have stimulatory effect on AMF variables, and minimum soil K is often prerequisite for mycorrhizal colonization in some plant species (Gamage *et al.*, 2004). The effect of soil K on mycorrhizal fungi not only depends on its own availability, but also on the concentration of other exchangeable ions like Ca and Mg in soil.

A number of well-supported dependencies between abundances of certain AMF taxa and soil properties such as pH, soil fertility and texture but lack of effect of available soil P on AMF community profiles was reported by Jansa *et al.* (2014). Antune *et al.* (2012) reported that incessant severe nutrient deficiencies might promote AMF with increased capacity to cheat the host plant (*i.e.,* communities that include AMF that access C without providing the deficient resources). However, it is possible that these AMF can confer to their hosts other benefits different than nutrient uptake, for instance protection against pathogens (Wehner *et al.,* 2010). It has been reported that as the level of phosphorus available to plant increases, the amount also increases in tissues and AM fungi symbiosis become non-beneficial to plants (Grant *et al.*, 2005). AM spore population and per cent root colonization were also reported lower at higher P level, and infection developed under these conditions of higher availability might function parasitically without making any beneficial contribution to plant nutrient supply.

Micronutrient level in soil samples of litchi orchards is presented in Table 3. In majority of samples, Zn and S content was below critical limit, whereas Fe, Cu, Mn and B was found above critical limit for litchi. The results indicated that micronutrients in general did not affect spore density of AMF in rhizosphere (zero or a very weak linear correlation) but did affect root colonization in a negative linear manner, the effect was more prominent above critical limit of

micronutrients. The influence of each micronutrients is presented and discussed specifically.

Analysis of data indicated that Zn content around critical limit (0.78 ppm) in rhizospheric soil had a positive effect on spore density and root colonization and beyond this limit, it drastically affected both the parameters. When data was plotted on a scatter diagram, the slope of regression line was negative for both the parameters (r = -0.42 for spore density and -0.44 for root colonization). The R^2 value indicated that Zn was responsible for 18-19% of the observed variability in the aforesaid parameters of AMF association in litchi. Soil having Fe content between 5-12 ppm showed higher number of spores of AMF. Similar trend was observed with respect to root colonization by AMF. Regression analysis indicated that Fe content above critical limit was not conducive for mycorrhizal association in litchi as negative linear relationship (r = -0.44 for spore density and -0.49 for colonization) was evident. Out of 105 samples, Cu content in soil was above critical limit (0.53 ppm) in 103 samples. Data revealed that the level of Cu in soil had no influence on spore density, but negatively affected root colonization by AMF in litchi. It was evident that about 23% of the observed variability in root colonization could be explained by Cu content of rhizosphere soil.

Mn content in all the soil samples were above critical limit (3 ppm). Maximum number of spores and root colonization was observed between 5-8 ppm. Regression analysis revealed a similar effect of this micronutrient to that of Cu on spore density and root colonization. Mn level in soil had no influence on spore density, but negatively affected root colonization by AMF in litchi. It was evident that about 27% of the observed variability in root colonization could be explained by Mn content of rhizosphere soil. In majority of soil samples, B content was above critical limit (0.53) and had a positive effect on spore density of AMF, but a negative linear correlation was apparent with root colonization. Maximum number of spore was observed between 3-4 ppm, whereas maximum root colonization was observed between 0.5 to 4.0 ppm. Boron is essential for translocation of sugar in roots. Though mycorrhiza themselves do not utilize boron, if boron concentration in soil rises above a certain level, it may become toxic for roots. This may restrict the mycorrhizal activities and by doing so harm the plants indirectly or may cause them to die. Ortas and Akpinar (2006) reported negative effects of high soil B concentration on root colonization. Maximum number of spores and colonization of roots by AMF was observed at S concentration ranging between 1.88 to 10.0 ppm. However, data revealed that S content in rhizosphere soil had no effect on spore density and root colonization when plotted on scatter diagram. The 'r' value was 0.08 and -0.06 for spore density and root colonization, respectively.

With regard to biotic factors, it was evident that age of litchi tree had neither influenced spore density of AMF in soil nor root colonization by AMF when data was plotted as scatterdiagram, though a significant variation was observed in spore count and % colonization vis-à-vis age of trees. The mean number of spores per gram soil were 4.0, 7.4, 7.1, and 5.7, whereas mean % colonization were 52.2, 49.8, 54.4 and 57.8 in the tree age group <15 yr, 15-30 yr, 30-45 yr and >45 yr, respectively. Root colonization ranged from 23.3-73.3, 3.3-86.7, 10.0-90.0, and 33.3-86.7 % in tree age group <15 yr, 15-30 yr, 30-45 yr and >45 yr, respectively. Data also revealed that among the two widely grown cultivars (cv.), 'China' had higher root colonization than 'Shahi'. About 50%, trees of cv. 'China' had root colonization above 50% whereas only 29.4% trees of cv. 'Shahi' had root colonization above 50%. As far as management status is concerned, comparatively higher spore density and root colonization of AMF was observed in irrigated, well managed orchards, where application of fertilizers and manures etc. were done than in non-irrigated, poorly managed orchards. About 46% of samples from irrigated fields had spore count above 5 spores g^{-1} soil (maximum up to 22), while in non-irrigated conditions 41% samples had more than 5 spores g^{-1} soil (maximum up to 13). In irrigated fields, 37.4% root samples examined colonization of AMF above 50%, while in non-irrigated field it was only 28%. Sharma and Kothamasi (2015) reported that soil moisture functioned as an abiotic filter and affected AMF community assembly inside *Sorghum vulgare* roots by regulating AMF colonization and phylotype diversity. Roots of plants in flooded soil had lowest AMF diversity, whilst root AMF diversity was highest under soil moisture regime of 15-20 %. Although plant biomass was not affected, root P uptake was significantly influenced by soil moisture.

In conclusion, the results of the study indicated widespread occurrence of AMF in association with litchi tree. The spore abundance was a good indicator of soils' AMF potential. Though it was impossible to distinguish between biotic and abiotic factors affecting spore abundance and root colonization of AMF in natural conditions, studies provided a clear understanding of how soil nutrients, management status and other factors influenced AMF symbiosis with litchi. The effects produced by soil micronutrients was studied less, but the results presented showed its importance as a regulating factor on AMF colonization. Future research should address the interplay of exogenous and endogenous factors in AMF, in particular, how nutrients impinge on symbiotic signalling and on the subsequent cellular program in host cells.

Acknowledgments

The authors gratefully acknowledge the financial assistance under the project 'Application of Microorganisms

in Agriculture and Allied Sectors' (AMAAS) from Indian Council of Agricultural Research (ICAR), India. Heartfelt thanks are also due to the Director, NRCL for providing logistics and constant encouragement during research.

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