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MICROBIOLOGY

Micronutrients Status Under Different Tree Species Plantations in Entisol Soil and its Relationship with AMF Root Colonization

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ABSTRACT

Entisol soil dominates in Chhattisgarh which do not support any vegetation because of preponderance of granular iron oxides, and nutrients and water deficiencies and poor microbial activities. In the present study secondary and micronutrients contents in entisol soil in 25 year old forest plantations comprising 09 different species have evaluated with its relationship on symbiotic *Arbuscular Mycorrhizal Fungi* (AMF) in roots for the screening of tree species suitable in entisol. The highest root colonization was 62% exhibited in *D. sissoo* and in *T. arjuna* while the lowest in *A. indica* (30%). Soil moisture ranged 10.03 to 12.3% varied significantly with different tree species at $P<0.01$. Sulfur and calcium content of soil found high in soil under *Eucalyptus globulus* plantation. Boron, Cu and Mn content were higher under *D. sisoo* and *T. arjuna* compared to other tree species plantations. A positive correlation recorded between AMF root colonization with different soil attributes viz. Mg (r^2 0.037), B (r^2 0.116), Cu (r^2 0.210), Fe (r^2 0.114), Mn (r^2 0.187) and Zn (r^2 0.119) was an indicative of the benefits of AMF to host tree species even in old plantations. Variations on tree species and soil parameters were observed significantly, however it was unpredictable to confirm that the particular tree species have better effects on the parameter of nutrients accumulated in rhizosphere soil and on the basis of AMF colonization in tree species plantations at this age.

Highlights

- Entisol soil is barren bhataland and its restoration as such is difficult due to presence of adverse soil properties.
- AMF symbiosis was higher in *D. sissoo*, *T. arjuna* and *Eucalyptus globulus* than other tree species indicates the relevance of symbiosis in entisol soil for managing sustainable plant demands for nutrients even after its establishment especially in forest species where usually no fertilizers are applied.
- The positive relationship with AMF root colonization and all the soil attributes depicts the important role of AMF for plantation program and should be encouraged as green technology for higher survival and establishment of forest plantation in wasteland soil.

Keywords: AMF, Micronutrient, Entisol soil, Tree plantation

Micronutrients nutrients including iron, zinc, manganese, magnesium and copper are very essential requires for enzyme activities and plant growth and development. In certain soil it is harder for plants to absorb these nutrients as may be present in forms that the plants cannot use. Moreover, plants often face significant challenges in obtaining an adequate supply of these nutrients to meet the demands due to their relative immobility. A deficiency of any one of them may result in decreased plant productivity and fertility (Morgan and Connolly 2013). To overcome nutrient limitation of the soil, plant evolves different mechanisms such as change in root architect that may increase the overall surface area of the root to increase nutrient acquisition through root proliferation (Lopez-Bucio *et al*. 2003). Plants are known to show different responses to different specific nutrient deficiencies and the responses can vary between species. In order to maintain nutrient homeostasis, plants must regulate nutrient uptake and must respond to changes in the soil as well as within the plant, many plant species forms mutually beneficial symbiotic relationships with soil-borne microorganisms. In these relationships, both the host plant and the microorganism symbiotically derive valuable resources that they need for their own productivity and survival as a result of the association.

AMF are ubiquitous in soil play pivotal role in managing nutrients under adverse, nutrient deficient and environmental stress conditions (Smith and Read 2008) after forming symbiosis. Time to time both the partners reviews and modifies the degree of symbiotic relationship depending upon the needs, results the outcome of the symbiosis varies (Johnson *et al*. 2003). The dependency of plant to AMF are usually high during initial stage of its establishment as newly germinated plant unable to absorb balance nutrition readily from soil due to restricted root system. In another hand well-established plant enable to uptake the demanded nutrients easily from soil, the degree of symbiosis formed poor and even at a parasitic level. Under these stages, the interaction between host and fungus depends especially upon the demand of particular elements by plant and its availability in soil. As different plants remove different amounts of mineral nutrients from the soil (Tulu 2002), thus deplete the soil nutrients in varying quantities and also plant exhibit varied degree of symbiotic bonds with AMF.

Majority of the study confined depicting the AMF symbiosis at different levels of N and P status in soil, while other elements also influence the activity of root colonization as plant reliant on balance nutrition. AMF have been reported to increase N, K, Ca, Zn or Cu uptake (Nikolaou *et al*. 2003), but it is unclear whether uptake of these nutrients are due to improved P nutrition. In order to explore the potential of green bio-fertilizer technology for sustainable restoration of wasteland, it is essential to understand the different factors influencing the degree of root symbiosis in particular soil types. Though many workers has done such work using AMF in various soil and hosts especially on macronutrient point of view, while the information's on micronutrient and AMF status in entisol soil is not available. Therefore, the present study was undertaken with objective to establish the relationship with secondary and micronutrients in entisol soil with AMF root colonization in different tree plantations.

MATERIAL AND METHODS

Chhattisgarh state has considerable extent of bhata soil commonly known as murrum soil and is categorized as entisol soil. This soil is nutritionally impoverished and has poor in water holding capacity due to preponderance of hard nodules of iron oxides. About 25 year before Nine tree species *Albizia lebbeck*, *Azadirachta indica*, *Dalbergia sissoo*, *Delonix regia*, *Eucalyptus globulous*, *Phyllanthus indica*, *Terminalia arjuna*, *Peltophorum ferruginium* and *Millettia pinnata* were tried at 4 × 4m distance in blocks in this soil near Bilaspur city, Chhattisgarh, India. This site was considered in the present investigation and soil and root samples from all the nine tree species were collected during March - April, 2016 by digging 3 pits of 30 cm^3 within plantation.

Root colonization by AMF represent the degree of symbiosis in host was determined as per in washed and chopped root of 1 cm size, followed heated in 10% KOH solution for 20 Minutes at 90°C and deeply pigmented roots were treated in 3% hydrogen per-oxide at room temperature for 5 minutes then acidified with in 1% HCL solution for overnight and stained with 0.05% trypan blue solution (1:1:1; water: glycerol: lactic acid) further heated at 80-85°C for 10 minutes as per Philips and Hayman, (1970). Over stained roots were kept with 50% glycerol solution to remove excess stain and finally examined under compound microscopic Leica DM 2500. A total of 5 segments of each root sample were mounted on the microscopic slide with 50% glycerol and smashed softly after placing a cover glass on the root pieces. Root samples were observed by a compound microscope at 200 magnifications. The percentage root colonization was calculated by following the method described by Mc Gonigle *et al.* (1990). The presence of mycelium was recorded as AM positive and total mycelia colonization was treated as percentage root colonization using following formula:

Root % colonization =

$$\frac{\text{Number of AFM positive segments}}{\text{Total number of segment observeved}} \times 100$$

AB-DTPA extractable Fe, Zn, Cu, Mg, B, S, Mn and heavy metals were analyzed following the method of Soltanpour and Schawab (1977) and then quantified using atomic absorption spectrophotometer. Soil moisture content was determined by drying 100g of freshly collected soil sample at 105^{o}C for 24 hrs in a hot air oven.

Standard errors of means were calculated for all the parameter studied and for F value Duncan Multiple Range test was used for mean comparison at 5% P level. Pearson coefficient was employed to determine the relationship between AMF colonization and soil physicochemical properties using SPSS 16.0.

RESULTS AND DISCUSSION

Root colonization by AMF was significantly highest in *Dalbergia sissoo* (62±5.15) and *Terminalia arjuna* (62±8.00) and lowest in *Azadirachta indica* (30±3.16) (Fig. 1A). It was recorded that the majority of the tree species used for plantation in entisol soil showed root infected > 50%, indicates both the existence and needs of symbiosis in these tree species. The rate of colonization in roots significantly varied between tree species as also reported by Tulu, (2002) as a result of differences in removal rate of nutrients by plants from the soil and thus its depletion in varying quantities resulted in a varied degree of root colonization with AMF. This results also accentuated with the finding of Jansa *et al*. (2002) depicts that the changes in tree species and host effects the rate of AMF colonization because of the variation in demand of nutrients by

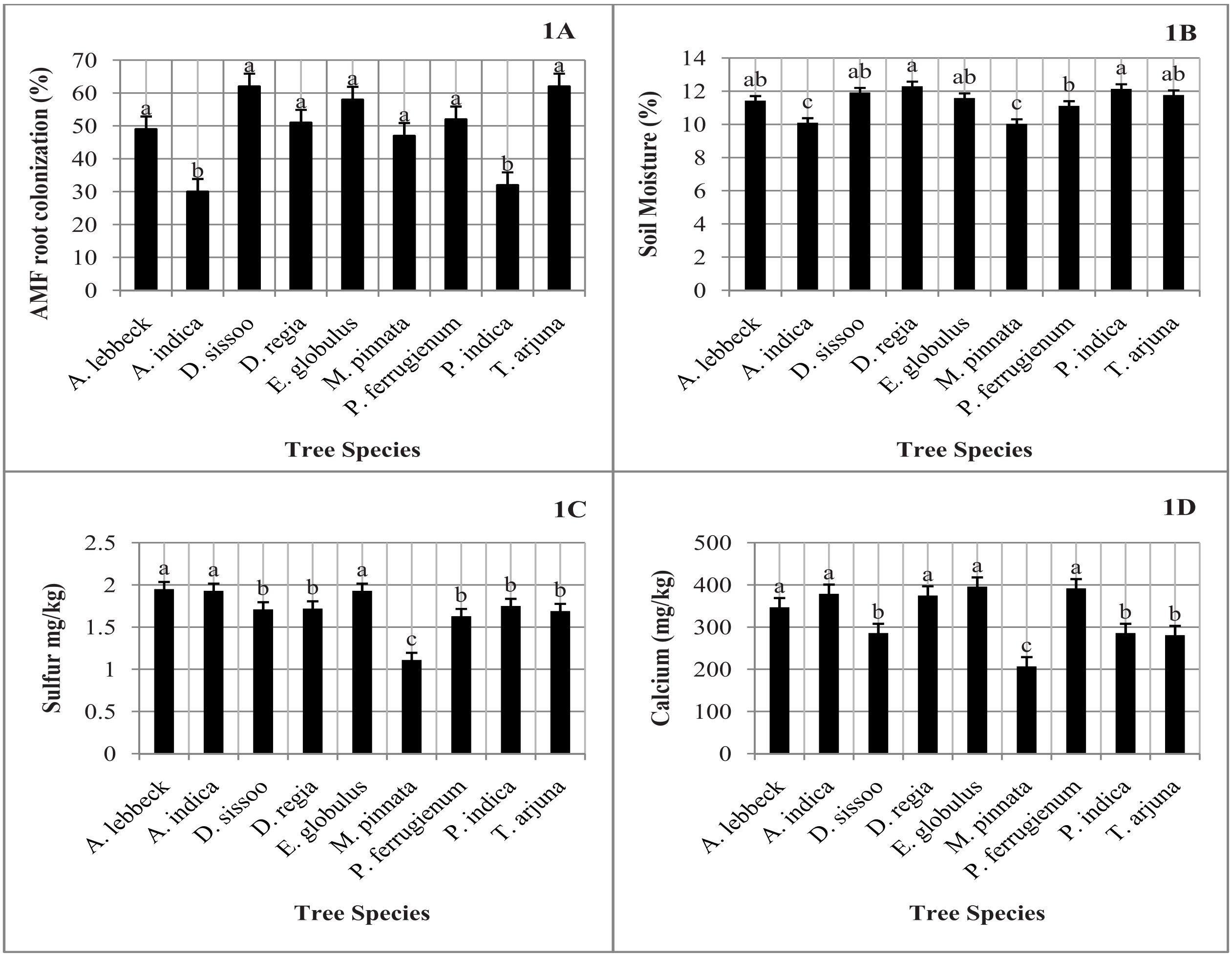


Fig. 1: (1A) AMF root infection (1B) Soil Moisture percent (1C) Sulfur and (1D) Calcium content in soil under different tree species planted in entisol soil. Data (means ± SE, n = 5) followed by different letters indicate significant differences between treatments (Tree species) at P < 0.05 by the Duncan's Multiple Range test

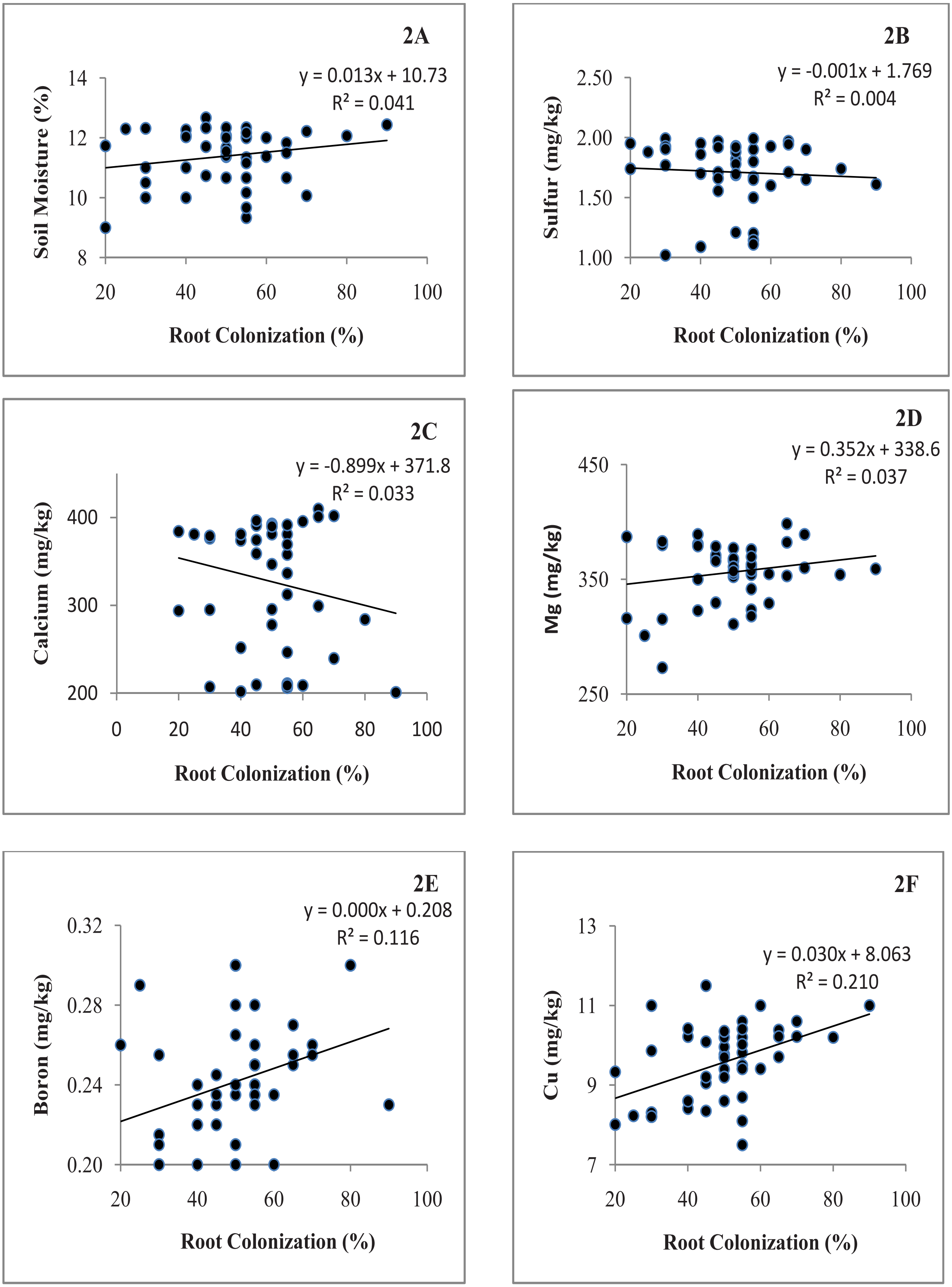
Table 1: Soil chemistry of tree plantations of 25 year age in entisol soil at Bilaspur, C.G. India. Data (means ± SE, n = 5) followed by different letters indicate significant differences between treatments (Tree species) at P < 0.05 by the Duncan's Multiple Range test

Tree Species	Mg (mg-kg^{-1})	B (mg-kg^{-1})	Cu (mg-kg^{-1})	Fe (mg-kg^{-1})	Mn (mg-kg^{-1})	Zn (mg-kg^{-1})
A. lebbeck	356 (±9.65)^{ab}	0.24 (±0.01)^{abc}	9.35 (±0.26)^{c}	6.62 (±0.06)^{b}	2.18 (±0.27)^{bc}	1.69 (±0.14)^{abc}
A. indica	382 (±1.41)^{a}	0.21 (±0.01)c	8.23 (±0.07)^{d}	6.92 (±0.05)^{ab}	2.06 (±0.20)^{c}	1.86 (±0.02)^{a}
D. sissoo	351 (±7.95)^{ab}	0.26 (±0.02)^{ab}	10.4 (±0.20)^{a}	5.25 (±0.30)^{c}	5.06 (±0.63)^{a}	1.60 9±0.05)^{abc}
D. regia	362 (±3.02)^{ab}	0.25 (±0.01)^{abc}	8.37 (±0.29)^{d}	6.45 (±0.17)^{b}	2.11 (±0.01)^{c}	1.56 (±0.12)^{bc}
E. globulus	363 (±14.9)^{ab}	0.24 (±0.01)^{abc}	9.43 (±0.27)^{bc}	6.60 (±0.06)^{b}	2.97 (±0.27)^{bc}	1.52 (±0.11)^{c}
M. pinnata	344 (±21.4)^{bc}	0.24 (±0.02)^{abc}	10.3 (±0.20)^{ab}	7.47 (±0.18)^{a}	3.89 (±0.03)^{ab}	1.11 (±0.03)^{a}
P. ferrugienum	371 (±6.48)^{ab}	0.23 (±0.01)^{bc}	9.66 (±0.30)^{abc}	6.55 (±0.07)^{b}	2.98 (±0.30)^{bc}	1.50 (±0.07)^{c}
P. indica	317 (±4.74)^{c}	0.25 (±0.01)^{ab}	9.87 (±0.55)^{abc}	5.01 (±0.30)^{c}	4.70 (±0.80)^{a}	1.87 (±0.03)^{ab}
T. arjuna	358 (±1.08)^{ab}	0.27 (±0.01)^{a}	10.4 (±0.22)^{a}	4.75 (±0.37)^{c}	5.12 (±0.62)^{a}	1.58 (±0.06)^{bc}
F value	3.326	2.287	7.933	20.159	7.717	7.097
Significance Level	0.006	0.043	0.001	0.001	0.001	0.001

host and to changes in soil nutrients and microbial activity. The AMF colonization in present study showed significant difference (F. 6.076, *P* = 0.001) ranged 20.0 to 90.0% at 95% confidence limit was an indicative of a changing physico-chemical state of the soil which directly affected on the symbiotic relationship between the plant and fungus as also ascribed by Aliasgharzadeh *et al*. (2001). Kim *et al*. (2017) has also concluded soil characteristics as a major factor influencing the colonization rates in the different soil types.

Soil moisture was found to be one of the important factor affect the AMF colonization in entisol soil. Its value differed significantly under tree species plantations at P <0.01, ranged between minimum 10.03 to a maximum of 12.30%. The highest percentage of moisture recorded in rhizospheric soil of *D. sissoo*, while the lowest under *M. pinnata* plantation. Other species viz *P. indica*, *D.regia*, *E. globulus* and *A. lebbeck* also found to retain moisture higher than open site but were observed statistically at par within tree species (Fig 1B). The moisture gradient between highest and the lowest recorded 2.27%. A weak positive correlation observed between root colonization and moisture percent (r^2 0.041) (Fig 2A) depicts that 40 to 60% roots were colonized by AMF at moisture range between11 to 12%, while the colonization rate dropped substantially below 10% soil moisture. This result indicates that certain level of soil moisture helps AMF establishment in host root and may be the influence of AMF at different moisture regime supports growth and development of host at varying rates. Deepika and Kothamasi (2015) have accentuated that moisture regulates AMF inside plant roots and at an optional soil moisture regime (15- 20%) supported higher root colonization following decreasing with increasing aridity in soil. Variation in moisture relations in soil is attributed to size of the plant as larger plants with larger root system have access to more extensive soil water reserve (Koide 1993) and so plant size effects often particularly evident in drying soils as in case of present study. In addition, the role of AM plants on water access compared to non AM plants and the characteristics of soil have been ascribed by (Hamblin 1985; Auge 2001; Auge 2004).

Sulfur found highest in soil under *Albizia lebbeck* (1.95±0.02 ppm) followed by *Azadirachta indica, E. globulus* (1.93±0.02 ppm) and the lowest under *Millettia pinnata* (1.11±0.03 ppm) (Fig 1C). The coefficient correlation between sulfur and root colonization found very weak (r^2= 0.004) indicates no role of AMF especially in old plantations for their sulfur demand (Fig 2B) but Mohamed *et al*., (2014) have reported positive effects of AMF on sulfur uptake by plants in pot experiment. Allen and Hill (2009) reported Sulfate was taken up by the fungus and transferred to mycorrhizal roots, increasing root S contents by 25% in a moderate concentration of sulfate. Calcium is one of the vital


2A
y = 0.013x + 10.73
R² = 0.041
Soil Moisture (%)
Root Colonization (%)
2B
y = -0.001x + 1.769
R² = 0.004
Sulfur (mg/kg)
Root Colonization (%)
2C
y = -0.899x + 371.8
R² = 0.033
Calcium (mg/kg)
Root Colonization (%)
2D
y = 0.352x + 338.6
R² = 0.037
Mg (mg/kg)
Root Colonization (%)
2E
y = 0.000x + 0.208
R² = 0.116
Boron (mg/kg)
Root Colonization (%)
2F
y = 0.030x + 8.063
R² = 0.210
Cu (mg/kg)
Root Colonization (%)

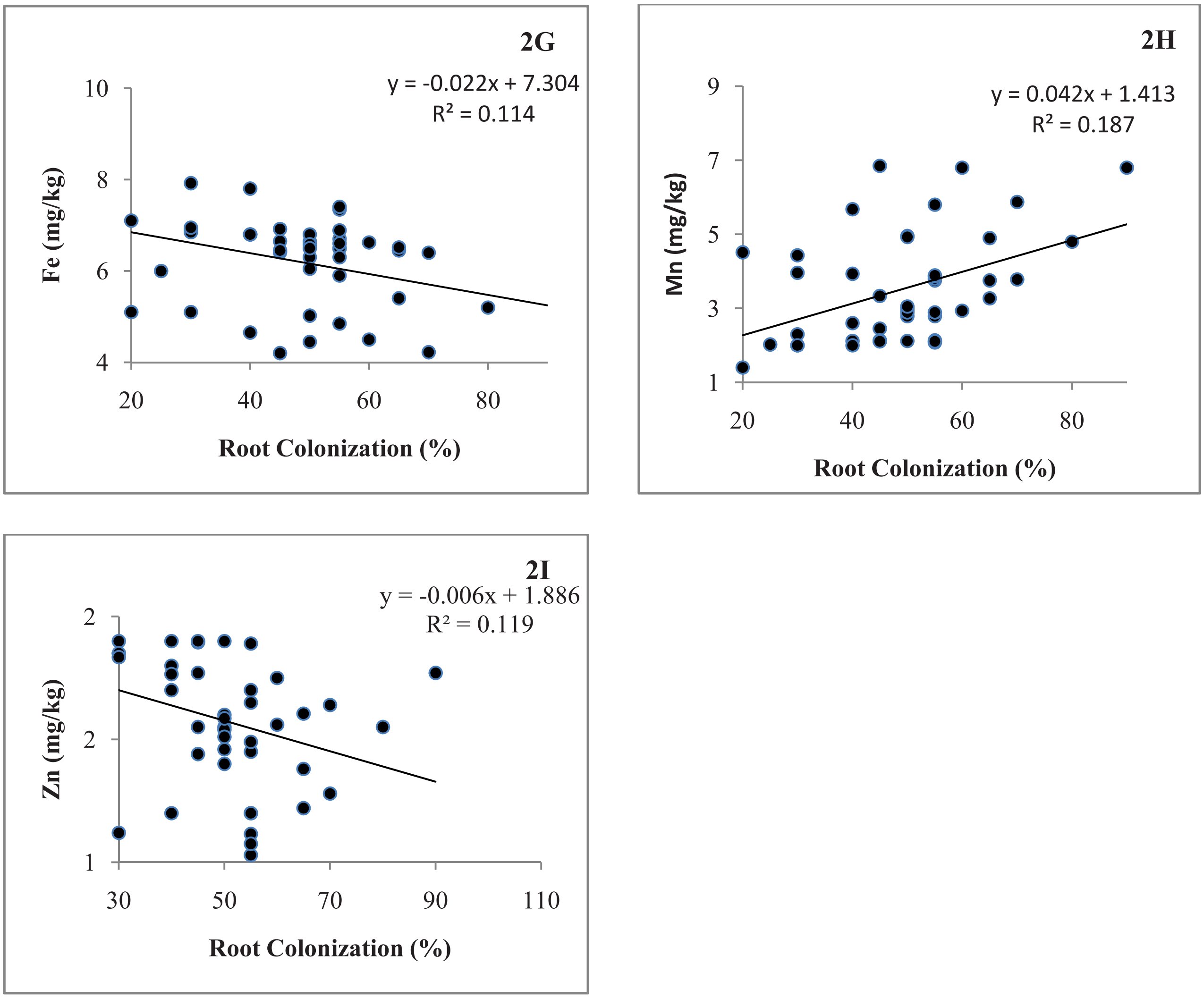


Fig. 2: Pearson Coefficient correlation (r^2) between AMF root colonization (RC) Vs(2A) Percent soil moisture(2B) Sulfur (2C) Calcium (2D) Magnesium (2E) Boron (2F) Copper (2G) Iron (2D) Manganese (2I) Zinc status of entisol soil

nutrients for wood forming tree species found between 201 to 406 ppm under different tree plantations (Fig 1D). The highest Ca found under *Eucalyptus globulus* plantation (396±4.96 mg kg^{-1}) followed by *Peltophorum ferrugienum* (392±3.48 mg kg^{-1}) and lowest in *Millettia pinnata* (207±1.50 mg kg^{-1}). Ca content in soil follows trends *E. globulus* > *P. ferrugienum* > *A. indica* > *D. regia* > *A. lebbeck* > *D. sissoo* > *P. indica* > *T. arjuna* > *M. pinnata*. AMF colonized plants found to affect Ca in soil positively (r^2 0.033) (Fig 2C) depicts the role of AMF on higher rate of Ca availability in rhizosphere of soil.

Micronutrients viz. Mg, B, Cu, Fe, Mn and Zn varied significantly among tree species plantations in entisol soil (Table 1). Mg content in soil found highest 382 mg kg^{-1} under *A. indica* and lowest 317 mg kg^{-1} under floor soil of *P. indica*. Boron in entisol soil found low compared to critical limit (0.53mg kg^{-1}). In present investigation the Cu content ranged 8.23 to 10.4 mg kg^{-1} and was highest under *D. sissoo* and *T. arjuna* while lowest under *A. indica* plantation. Fe found in low concentration compared to the critical limit (7.0 mg kg^{-1}) except under *M. pinnata*.. Mn also found in the range 2.06 to 5.12 mg kg^{-1} lowest under *A. indica* and highest under *T. arjuna* plantation. Zn found highest 1.87 mg kg^{-1} under *P. indica* and lowest 1.11 mg kg^{-1} under *M. pinnata*. It is clear that micronutrients contents varied in soil under tree species plantation significantly and no apparent relationship established between AMF and micro nutrient contents in soil. However, weak but significant positive correlation found between

AMF root colonization and soil attributes (Fig 2D – 2I) viz. Mg (r^2 0.037), B (r^2 0.116), Cu (r^2 0.210), Fe (r^2 0.114), Mn (r^2 0.187) and Zn (r^2 0.119). This is an indicative that plant interacts with AMF for continuous supply of desired nutrients beyond depletion zone even in older plantations. AMF has described to lack host and niche-specificity, and therefore develops in a wide range of plants and environmental conditions therefore; apparent role of AMF is unpredictable always since different plant species vary their response to the same AMF species. This results was an agreement with other workers who have reported that AM symbiosis improves the S nutrition (Giovannetti *et al*. 2014), Zn (Lehmann *et al*. 2014), Cu, Fe and Mn (Lehmann and Rillig 2015) and AM fungal field inoculation could be an effective to improve productivity of soil. Recently Nouri *et al*. (2014) has tested several elements to verify the effects on AM development and their nutrient-dependent regulation and propounded that it provides an important feedback mechanism for plants to promote or limit fungal colonization according to their needs. Their results have shown that nitrate can potentially exert negative regulation on AM, while sulfate, Mg, Ca, and Fe have no effect. The nutrient starvation of plant triggers a dominant AM-promoting signal and symbiosis takes place between the partners. In addition, Walder and van der Heijden (2015) have reported that the cooperation in AM interactions is related to the partners involved in the symbiosis, and depends on physic-chemical properties of soil (Nongkling and Kayang 2017), environmental conditions and acquisition of surplus resources.

Conclusively, AMF root colonization in all the tree plantations even after its 25th year of age indicates overall positive outcomes for plant due to the several nutrition-related benefits. The varying degree of AMF root colonization and soil attributes especially soil moisture and micro nutrients might be the results of variation in the rate of nutrient demanded by host and its supply from the soil. We found weak but positive correlation between AMF root colonization and with almost all parameters of present study clearly envisages that these tree species still maintained symbiosis with AMF for efficient nutrition and biomass development under nutrient deficient and dried entisol soil. In open field condition where normally number of other factors together affects the AMF, the next significant step toward the stable use of AMF in forestry is to carry out large-scale multi-location field trials and conduct cost-benefit analyses in order to increase awareness among the potential end-users of the benefits of AMF for better survival and establishment of forestry plantation.

REFERENCES

Aliasgharzadeh, N., Rastin, S.N., Towfighi, H. and Alizadeh, A. 2001. Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza*, **11**: 119-122.

Allen, James W. and Hill, Y S. 2009. Sulfur Transfer through an Arbuscular mycorrhiza. *Plant Physiol*., **149**(1): 549–560.

Auge, R.M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, **11**: 3-42.

Auge, Robert M. 2004. *Arbuscular mycorrhizae* and soil/plant water relations. *Canadian Journal of Soil Science*, **84**(4): 373-381.

Deepika, S. and Kothamasi, D. 2015. Soil moisture a regulator of *Arbuscular mycorrhizal* fungal community assembly and symbiotic phosphorus uptake. *Mycorrhiza*, **25**(1): 67-75.

Giovannetti, M., Tolosano, M., Volpe, V., Kopriva, S. and Bonfante, P. 2014. Identification and functional characterization of a sulfate transporter induced by both sulfur starvation and mycorrhiza formation in *Lotus japonicus*. *New Phytol*., **204**: 609-619.

Hamblin, A.P. 1985. The influence of soil structure on water movement, crop root growth and water uptake. *Adv. Agron*., **38**: 95-158.

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R. and Frossard, E. 2002. Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza*, **12**: 225-234.

Johnson, N.C., Rowland, D.L., Corkidi, L., Egerton-Warburton, L.M. and Allen, E.B. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology*, **84**: 1895-1900.

Koide, R .1993. Physiology of the mycorrhizal plant. *Adv Plant Pathol*., **9**: 33-54.

Kim, S.J., Eo, Ju-Kyeong, Lee, E.H., Park, H. and Eom, A.H. 2017. Effects of Arbuscular mycorrhizal Fungi and Soil Conditions on Crop Plant Growth. *Mycobiology*, **45**(1): 20-24.

Lehmann, A. and Rillig, M.C. 2015. Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops -A meta-analysis. *Soil Biol. Biochem*., **81**: 147-158.

Lehmann, A., Veresoglou, S.D., Leifheit, E.F., Rillig, M.C. 2014. *Arbuscular mycorrhizal* influence on zinc nutrition in crop plants - A meta-analysis. *Soil Biol. Biochem*., **69**: 123-131.

Lopez-Bucio J., Cruz-Ramírez, A. and Herrera-Estrella, L. 2003. The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology,* **6**: 280-287.

Mc Gonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. and Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytol*., **115**: 495-501.

Mohamed, Amal A., Ewedab, Wedad E.E., HeggoaEnas, A.M. and Hassan, A. 2014. Effect of dual inoculation with arbuscular mycorrhizal fungi and sulphur-oxidising bacteria on onion (*Allium cepa* L.) and maize (*Zea mays* L.) grown in sandy soil under greenhouse conditions. *Annals of Agricultural Sciences,* **59**(1): 109-118.

Nikolaou, N.A., Koukourikou, M., Angelopoulos, K. and Karagiannidis, N. 2003. Cytokinin content and water relations of Cabernet Sauvignon grapevine exposed to drought stress. *J. Hortic. Sci. Biotechnol.,* **78**: 113-118.

Nongkling, P. and Kayang, H. 2017. Soil physicochemical properties and its relationship with AMF spore density under two cropping systems. *Current Research in Environmental and Applied Mycology,* **7**(1): 33-39.

Nouri, E., Breuillin-Sessoms, F., Feller, U. and Reinhardt, D. 2014. Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in *Petunia hybrida*. *PLoS ONE,* **9**: e90841.

Morgan, J.B. and Connolly, E.L. 2013. Plant soil interactions: Nutrient uptake. *Nature Education Knowledge,* **4**(8): 2.

Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots for rapid assessment of infection. *Transactions of British Mycological Society,* **55**: 158-161.

Smith, S.E. and Read, D.J. 2008. Mycorrhizal symbiosis, 3rd edn. Academic Press: Amsterdam, the Netherlands and Boston, MA, USA.

Soltanpour, P.N. and Schwab, A.P. 1977. A new soil test for simultaneous extraction of macro and micronutrient in alkaline soils. *Commun. Soil Sci. Plant Anal.,* **8**: 195-207.

Tulu, T. 2002. Soil and water conservation for sustainable agriculture. Mega publication enterprise, Addis Ababa, Ethiopia.

Walder, F. and van der Heijden, M.G.A. 2015. Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. *Nat. Plants,* **1**: 15159.