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Seasonal Dynamics of Arbuscular Mycorrhizal Fungi from Iron Ore Mine Wastelands of Goa, India

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ABSTRACT

Arbuscular mycorrhizal fungal association in relation to edaphic and climatic factors was assessed in eight plant species *viz*., *Chromolaena odoratum*, *Emilia sonchifolia*, *Mimosa pudica*, *Ludwigia parviflora*, *Ischaemum semisagittatum*, *Acacia auriculiformis*, *Acacia mangium*, and *Trema orientalis* for one year from Codli iron ore mine reject dump in Goa. Arbuscular mycorrhizal (AM) colonization levels and spore numbers varied significantly between the plant species in the different seasons. The calculated correlation coefficient showed that soil moisture was negatively correlated to EC, N, P, K, calcium, organic carbon, and organic matter. Soil moisture had a positive influence on AM fungal colonization and a negative influence on spore density in all the plant species. Spore number was maximum in pre-monsoon and least in monsoon, while AM colonization was maximum in monsoon and least in pre-monsoon. A total of 40 AM fungal species belonging to 13 genera were reported during the study. Among the genera, the genus *Glomus* was dominant in the pre-monsoon, *Acaulospora* was dominant in the monsoon, and *Gigaspora* was dominant in the post-monsoon season.

Keywords: Arbuscular mycorrhizal fungi, Edaphic factors, Seasonal variation, Mine spoils.

INTRODUCTION

Widespread in their seasonal occurrence and distribution, AM fungi are a significant part of every natural and cultivated ecosystem, playing an essential role in plant species diversity and survival. It is well known that AM fungal symbiosis facilitates the survival, growth, and establishment of plants in extreme habitats (Apple, 2010; Yang *et al.,* 2017; Kumar *et al.,* 2010). Environmental conditions greatly influence the density and composition of AM fungi. Spore germination, hyphal spread within the soil, colonization levels, fungal survival, and growth promotion may respond differently to environmental conditions. Thus, it is challenging to predict the influence of environmental variables on mycorrhizal association in plants.

Studies on seasonal fluctuations in AM fungal communities in response to climatic and edaphic factors have been documented earlier (Alguacil *et al*., 2016; Halder *et al*., 2015; Deepika and Kothamasi 2015; Melo *et al.,* 2019). Similarly, studies on the occurrence of AM fungi from different mine spoils have been reported earlier (Sastry and Johri, 1999; Teixeira *et al*., 2017; Rodríguez *et al*., 2021; Bukhari and Rodrigues, 2022). These investigations have stressed the importance of AM fungal relationships in allowing successful recolonization and plant growth. There are several reports on the role of AM fungi in the revegetation of mining (Hazarika *et al.,* 2010, 2014; Agus *et al.,* 2019). It has been reported that soil disturbances associated with mining activity reduce AM fungal colonization in vegetation to different extents, depending upon the mining operation and environment (Agus *et al.,* 2019; Bukhari and Rodrigues, 2022).

Studies on seasonal dynamics of AM fungi concerning iron ore mine wastelands are very scarce. Hence, the present study was undertaken to understand the pattern of colonization, spore density, and species richness in relation to the seasonal changes in the climatic and edaphic factors in some plant species from iron ore mine wastelands at Codli, Goa.

MATERIALS AND METHODS

Study Site

The study was carried out on an iron ore mine site at Codli, Goa (15^0 20' 53" N Latitude and 74^0 8' 33" E Longitude) during pre-monsoon (March), monsoon (July) and post-monsoon (November) seasons. Six quadrats, each with an area of 10 x 10 sq. mt., were randomly laid at the site. Eight angiosperm plant species comprising five herbs *viz*., *Chromolaena odoratum* (L.) King & Robinson, *Emilia sonchifolia* (L.) DC., *Mimosa pudica* L., *Ludwigia parviflora* L., and *Ischaemum semisagittatum* Roxb. and three tree species *viz*., *Acacia auriculiformis* A. Cunn. ex Benth., *Acacia mangium* Willd., and *Trema orientalis* (L.) Blume common to all the six quadrates were taken up for study. Plants collected were identified using standard floras (Rao, 1985 & 1986; Mathew, 1992; Mohanan and Henry, 1994; Naithani *et al*., 1997).

Soil analysis

Soil temperature was recorded during pre-monsoon (March), monsoon (July), and post-monsoon (November) using a soil thermometer. For

analysing soil moisture, pH, EC, N, P, K, Ca, Mg, organic carbon (OC), organic matter, mine reject samples were collected from a depth of 0-25 cm from five different locations for each quadrate at the study site and were brought to the laboratory in polyethylene bags. Samples from each quadrate were passed through a 2mm sieve to remove the larger soil particles and were mixed thoroughly to obtain a composite sample. Later, each composite sample was processed three times to get the mean value. This was repeated for all six quadrates, and the average mean value was obtained for each quadrate.

Moisture content and soil pH were measured within 2-3 hours of sample collection. A known amount of soil was dried at 85^{o}C to a constant weight to determine the moisture content and expressed as a percentage of oven-dried weight. Soil pH was measured after dilution with distilled water (1:1 w/v soil: water), and Electrical Conductivity (EC) was determined in 1:1 water: waste extract using a conductivity meter (Bower and Wilcox, 1965). Soil nitrogen was determined by the micro-Kjeldahl method (Jackson, 1971). Soil P was determined by the molybdenum blue method (Jackson, 1971). Potassium was determined by the flame photometric method (Jackson, 1971). Exchangeable calcium magnesium was detected by flame emission spectrophotometry. Organic carbon and organic matter content were detected by Walkley and Black's rapid titration method (Jackson, 1971).

Sampling

Roots and rhizosphere soils were sampled individually for each plant species from all the six quadrates during pre-monsoon (March), monsoon (July), and post-monsoon (November) for one year. For trees, the roots were dug and traced back to the plant, which ensured that the roots belonged to the intended plant species. Samples of herbs were usually made by uprooting the entire plant. Plants were uprooted along with the rhizosphere soil and were brought to the laboratory in polyethylene bags. Plants were shaken to remove the adhering soil particles. Feeder roots were cut into 1cm bits and were processed for further studies.

Assessment of AM colonization and spore densities

Roots were observed under a stereomicroscope (x20) for AM fungal spores attached to roots. After examination, the roots were cut into 1 cm pieces, cleared in 10% KOH, acidified with 5N HCl, and stained with Trypan blue (Phillips and Hayman, 1970). The roots were kept overnight and immersed in the staining solution. Roots that remained dark after clearing were bleached in alkaline H_2O_2 before acidification. The stained roots were examined under a compound microscope (x200-400) for AM fungal structures. Percent root colonization was estimated using the magnified intersection method (McGonigle *et al.,* 1990).

Hundred grams of rhizosphere soil of each plant from each quadrate was assayed for spore count using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Estimation of spore density was carried out according to Gaur and Adholeya (1994). Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were transferred using a needle on a wet filter paper for establishing trap cultures or to polyvinyl alcohol-lactoglycerol (PVLG) (Koske and Tessier, 1983) with or without Melzer's reagent on a glass slide for identification.

Identification of AM fungal species

Intact and crushed spores in PVLG with or without Melzer's reagent were examined under a Leica compound microscope, identified based on spore morphology and sub-cellular characters, and compared with original descriptions. Spore colour was examined under a Leica stereomicroscope using intact spores immersed in water. AM spores were identified based on morpho-taxonomic criteria (Almeida and Schenck, 1990; Schüßler and Walker, 2010; Rodrigues and Muthukumar, 2009; Redecker *et al.,* 2013).

Establishment of Trap cultures

Trap cultures were established from fresh field soils collected from all four sites. The soil samples were mixed with autoclaved sand in a ratio of 1:2 (v/v) and filled in 15-cm diameter pots. Pots were planted with one of the four hosts: (i) *Eleusine coracana* (L.) Gaertn, (ii) *Solanum lycopersicum*. L. (iii) *Allium cepa* L. and (iv) *Plectranthus scutellarioides* (L.) R. Br. (Coleus). Pots were irrigated from the top when the top one cm of the surface became dry. After three months, the plants were cut back to the soil surface and reseeded with the same host. The plants were allowed to grow for an additional three months when they were sampled to determine AM fungal species. The spores isolated from the trap cultures were later used to confirm the identified spores recovered during the study period.

Statistical analysis

The data on AM colonization and AM fungal structures were arcsine square-root transformed, and spore numbers were log-transformed before statistical analysis. Analysis of Variance (ANOVA) on edaphic and mycorrhizal variables was carried out to investigate their variations with space and time. The variations within a year in edaphic variables and mycorrhizal status were examined for each species by ANOVA, and the means were separated using Duncan's Multiple Range Test (DMRT).

Three-way ANOVA was used to study whether the AM fungal colonization pattern and spore numbers were seasonal for the whole data set (all eight plant species). Pearson's correlation was performed using SPSS to assess the relationship between edaphic and mycorrhizal variables. The frequency of occurrence was calculated using the formula given below.

$$\text{Frequency (\%)} = \frac{\text{Number of samples containing a species}}{\text{Total number of samples examined}} \text{ X } 100$$

RESULTS

The results revealed that the soil was deficient in nutrients. A significant difference was observed in the soil characteristics during the different seasons **(Table 1).** Soil temperature was maximum during pre-monsoon (24 - 40^0C), followed by post-monsoon (22 - 35^0C), and was least during monsoon (20 - 28^0C).

Table 1: Seasonal changes in spoil characteristics at the study site.

Parameter	Pre-monsoon	Monsoon	Post-monsoon	F-ratio
SM (%)	3.100 a	11.87 c	7.517 b	**
pH	6.400 a	6.300 a	6.633 b	**
EC	0.1047 b	0.0775 a	0.1038 b	NS
N	60.33 c	41.50 b	30.83 a	**
P	117.83 c	93.833 a	102.50 b	**
K	40.83 c	27.33 a	31.167 b	**
Ca	5.94 b	3.867 a	5.338 b	**
Mg	1.573ab	1.327a	2.023b	NS
OC (%)	0.245 b	0.037 a	0.082 a	**
OM (%)	0.4220 b	0.065 a	0.30 b	**

Significant at ** - P< 0.01; NS, Not significant.
In a row means followed by same letter(s) are not significantly different according to DMRT (P< 0.05)

Mycorrhizal colonization and spore number

Mycorrhizal colonization was maximum in *Mimosa pudica* (46.40%) and was least in *Acacia mangium* (23.56%). A similar trend was observed for mean hyphal, arbuscular, and vesicular colonization. The root length colonized by hyphae, vesicles, and arbuscules exhibited variation between plant species. The mean spore number was higher in *Chromolaena odoratum* (172 spores 100 g^{-1}), *Mimosa pudica* (140 spores 100 g^{-1}), *Trema orientalis* (136 spores 100 g^{-1}) and *Ischaemum semisagittatum* (115 spores 100 g^{-1}) compared to *Ludwigia parviflora* (73 spores 100 g^{-1}), *Acacia auriculiformis* (63 spores 100 g^{-1}), and *Acacia mangium* (54 spores 100 g^{-1}) for all the three seasons **(Figure 1).**

Pattern of mycorrhizal colonization and AM fungal structures

Mean colonization levels and the root length colonized by hyphae, arbuscules, vesicles, and total root length colonized varied significantly between the seasons (**Figure 2-5)**. Three-way ANOVA on mycorrhizal colonization and AM fungal structures indicated significant variation in the colonization levels and structures between species, seasons, and species x seasons (**Table 2**).

A significant species x seasonal interaction for AM colonization and structures indicates that different plant species exhibit different patterns of AM colonization and AM fungal structures. Varied patterns of mycorrhizal colonization were recorded for different seasons. All the plant species studied showed high levels of hyphal and total root length colonization in monsoon followed by post-monsoon, while the colonization levels were least during pre-monsoon. Arbuscular colonization levels in all the plant species except *A. auriculiformis* were maximum during monsoon followed by post-monsoon, while the colonization levels were least during pre-monsoon. In *A. auriculiformis,* arbuscular colonization was maximum during post-monsoon, followed by monsoon, and was least during pre-monsoon. The vesicular colonization levels varied for all the plant species and varied for different seasons (**Figure 2-5)**.

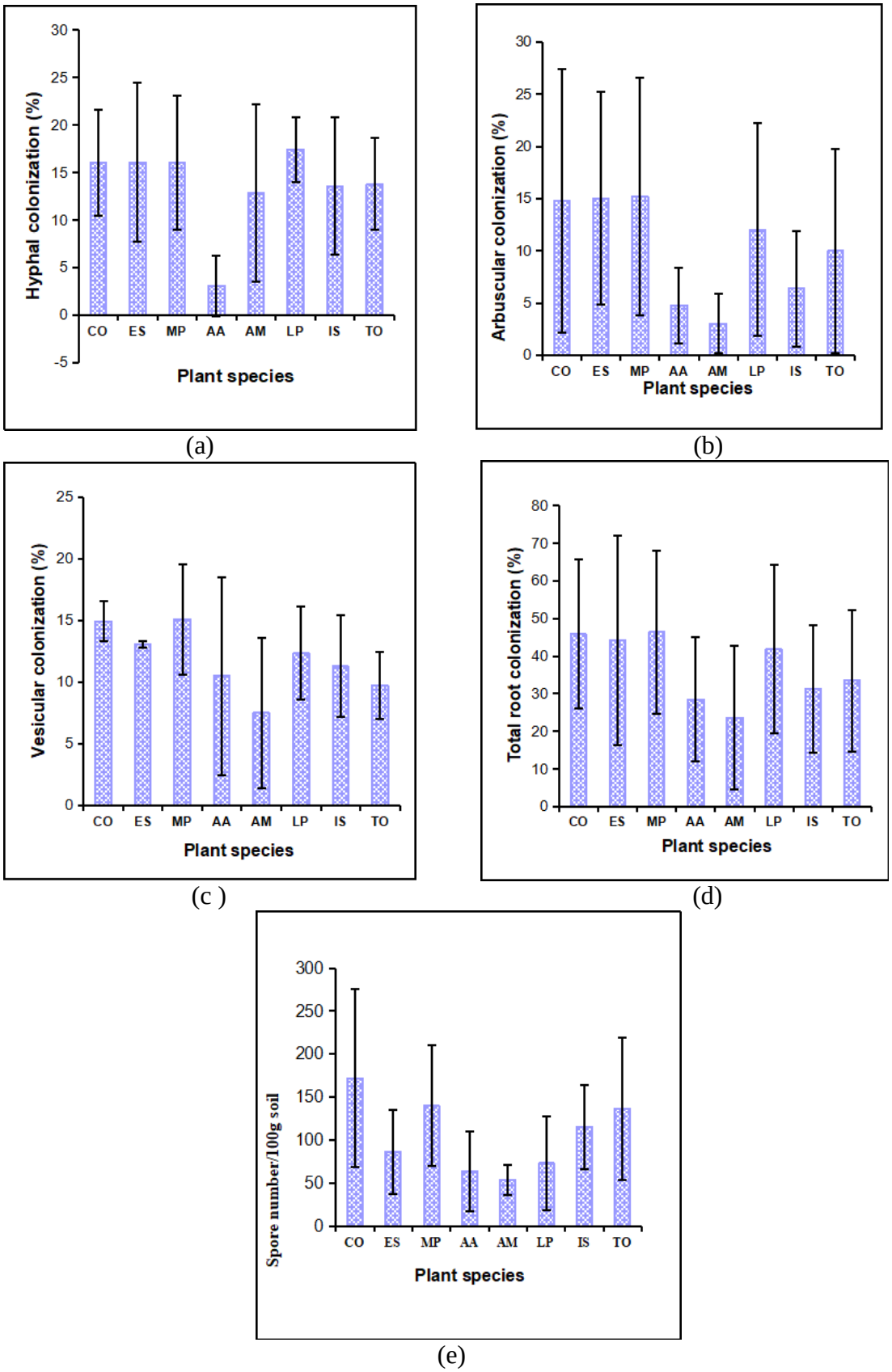

Figure 1: Mean AM root colonization (a-d) and spore number (e) in *Chromolaena odoratum* (CO), *Emilia sonchifolia* (ES), *Mimosa pudica* (MP), *Acacia auriculiformis* (AA), *Acacia mangium* (AM), *Ludwigia parviflora* (LP), *Ischaemum semisagittatum* (IS) and *Trema orientalis* (TO). Error bar indicates ± 1 SD.

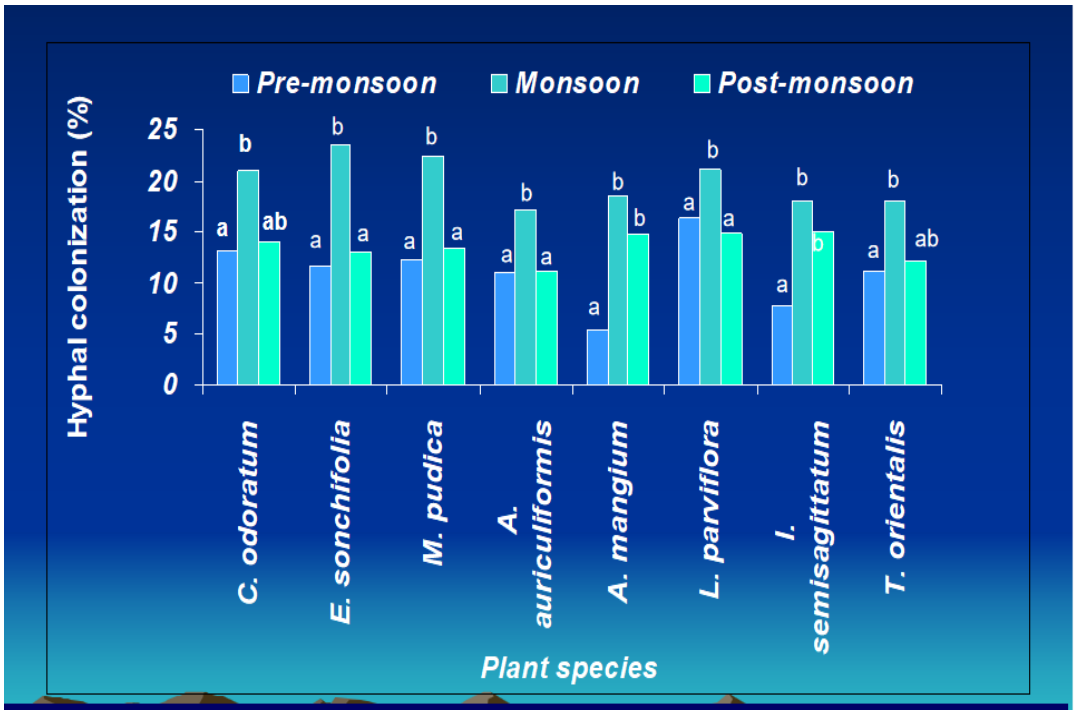

Figure 2: Mean hyphal colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter (s) is not significant according to DMRT (P<0.05). *C.=Chromolaena; E.= Emilia; M.=Mimosa; A.=Acacia; L.=Ludwigia; I.=Ischaemum; T.=Trema.*

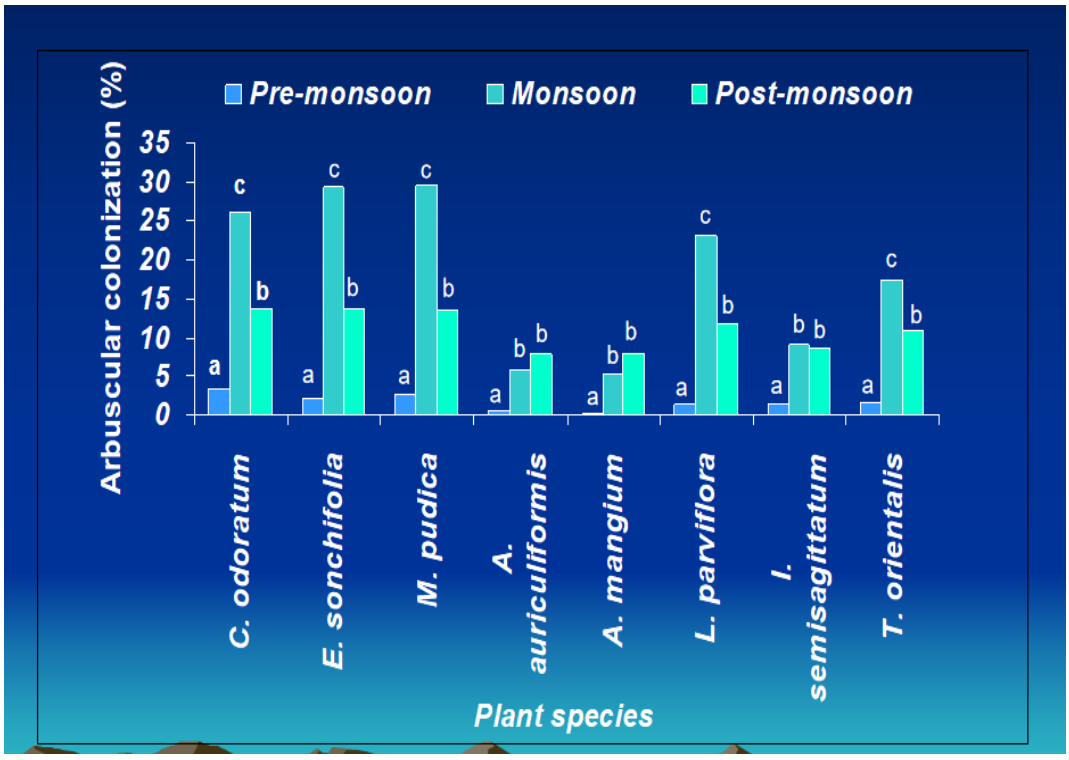

Figure 3: Mean arbuscular colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter (s) is not significant according to DMRT (P<0.05). *C.= Chromolaena; E.=Emilia; M.=Mimosa; A.=Acacia; L.=Ludwigia; I.=Ischaemum; T.=Trema.*

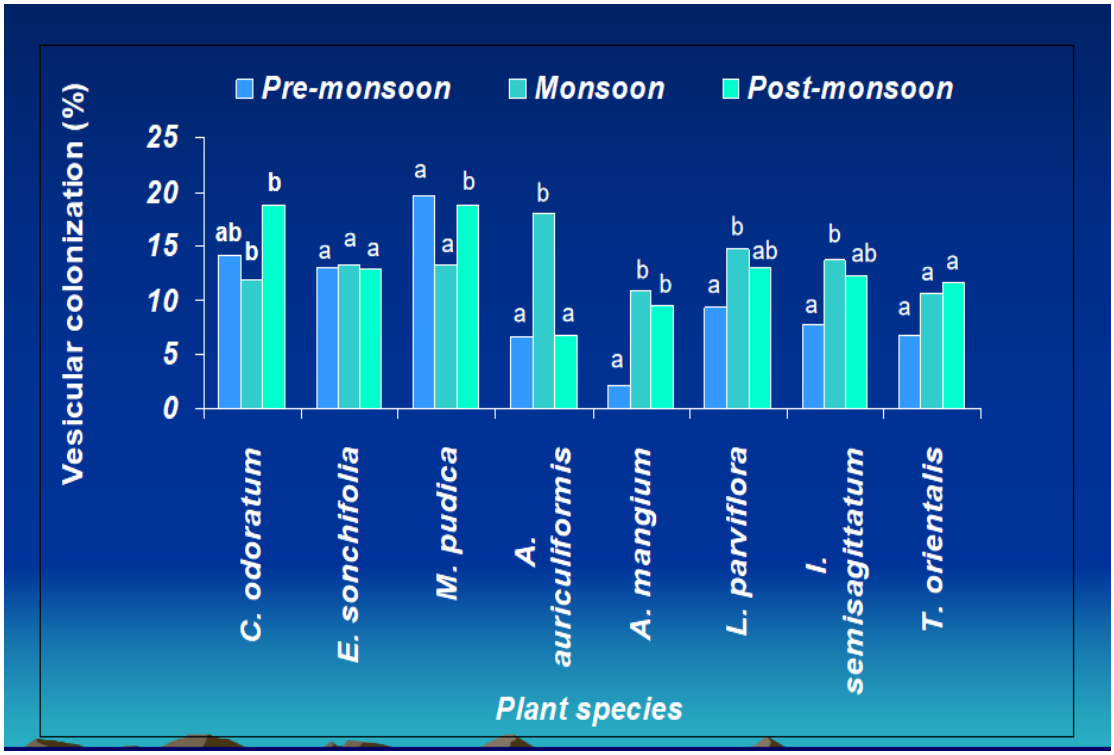

Figure 4: Mean vesicular colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter (s) is not significant according to DMRT ($P<0.05$). *C.=Chromolaena; E.=Emilia; M.=Mimosa; A.=Acacia; L.=Ludwigia; I.=Ischaemum; T.=Trema.*

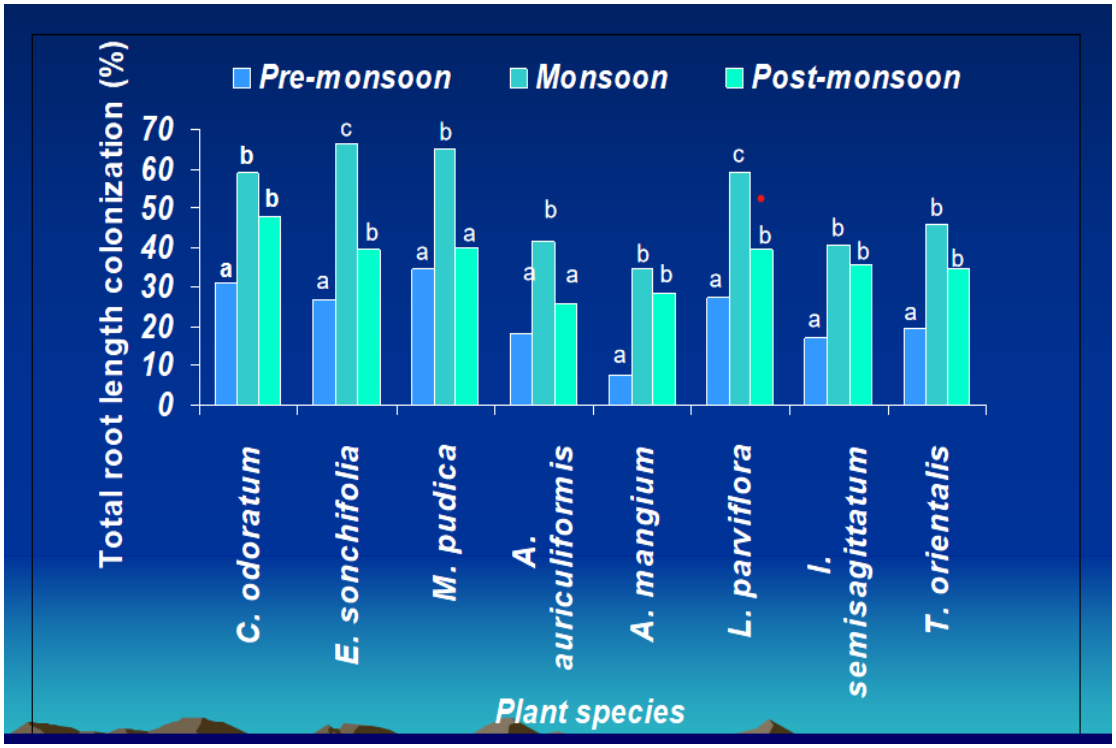

Figure 5: Mean total root length colonization in plant species during pre-monsoon, monsoon and post-monsoon. Columns of a season followed by same letter (s) is not significant according to DMRT ($P<0.05$). *C.=Chromolaena; E.=Emilia; M.=Mimosa; A.=Acacia; L.=Ludwigia; I.=Ischaemum; T.=Trema.*

Table 2: Three-way analysis of variance (ANOVA) of the data on AM fungal structures and total colonization.

		d.f.	F ratio	Significance
Plant Species (P)		7		
	Hyphae		2.70	*
	Arbuscules		23.76	**
	Vesicles		7.14	**
	Total		19.90	**
Season (S)		2		
	Hyphae		47.17	**
	Arbuscules		195.98	**
	Vesicles		8.98	**
	Total		136.13	**
P x S		14		
	Hyphae		1.66	NS
	Arbuscules		2.94	**
	Vesicles		4.06	**
	Total		1.78	*

Significant at * - P < 0.05; ** - P< 0.01; NS, Not significant; d.f., Degrees of freedom.

Relationship between AM fungal structures

Among the various AM fungal structures, a significant (P<0.05, 0.01) positive correlation between hyphal and arbuscular colonization was observed in *E. sonchifolia*, *M. pudica*, *A. mangium*, *I. semisagittatum*, and *T.orientalis*. Positive correlation between hyphal and vesicular colonization was recorded in *A. mangium* (P<0.001), *A. auriculiformis* (P<0.01), *I. semisagittatum* (P<0.01), and *T. orientalis* (P<0.05). A significant (P<0.01) positive correlation between hyphal and total root colonization was observed in *C. odoratum*, *M. pudica*, and *L. parviflora*. At the same time, it was highly significant (P<0.000) in *E. sonchifolia*, *A. auriculiformis*, *A. mangium*, *I. semisagittatum*, and *T. orientalis*. A highly significant (P<0.001, 0.000) positive correlation was noted between arbuscular and total root length colonization in all the plant species. A highly significant positive correlation existed between arbuscular and vesicular colonization in *A. mangium* (P< 0.000), *L. parviflora* (P< 0.01), and *I. semisagittatum* (P< 0.05). A highly significant (P< 0.01, 0.000) positive correlation between vesicular and total root colonization was recorded in *T. orientalis*, *A. auriculiformis*, *A. mangium*, *L. parviflora* and *I. semisagittatum* (**Table 3)**.

Table 3: Pearson correlation coefficient for AM fungal structures.

Plant species	H x A	H x V	H x T	A x V	A x T	V x T
Chromolaena odoratum	0.393	-0.263	+0.596**	-0.057	+0.913****	0.162
Emilia sonchifolia	+0.650**	-0.104	+0.798****	-0.010	+0.892****	0.289
Mimosa pudica	+0.488*	-0.353	+0.688**	-0.317	+0.849****	0.169
Acacia auriculiformis	0.362	+0.670**	+0.813****	0.395	+0.711***	+0.876****
Acacia mangium	+0.638**	+0.715***	+0.907****	+0.787****	+0.850****	+0.919****
Ludwigia parviflora	0.407	0.384	+0.635**	+0.617**	+0.917****	+0.794****
Ischaemum semisagittatum	+0.515*	+0.594**	+0.854****	+0.541*	+0.836****	+0.774****
Trema orientalis	+0.495*	+0.517*	+0.832****	0.298	+0.837****	+0.663**

Significant at * - P< 0.05; ** - P< 0.01; *** - P< 0.001; ***** - P< 0.000
H=Hypha; A=Arbuscule; V=Vesicle; T=Total root colonization.

Pattern of mycorrhizal spore number

Maximum spore density was recorded during pre-monsoon, followed by post-monsoon, and the least was recorded during the monsoon season. Mean spore density for individual plant species varied significantly between the seasons (**Figure 6**). There were highly significant differences in mean spore densities between species, seasons, and species x season. The significant species x season interaction (P<0.01) suggests the difference in seasonal pattern in spore number (**Table 4)**.

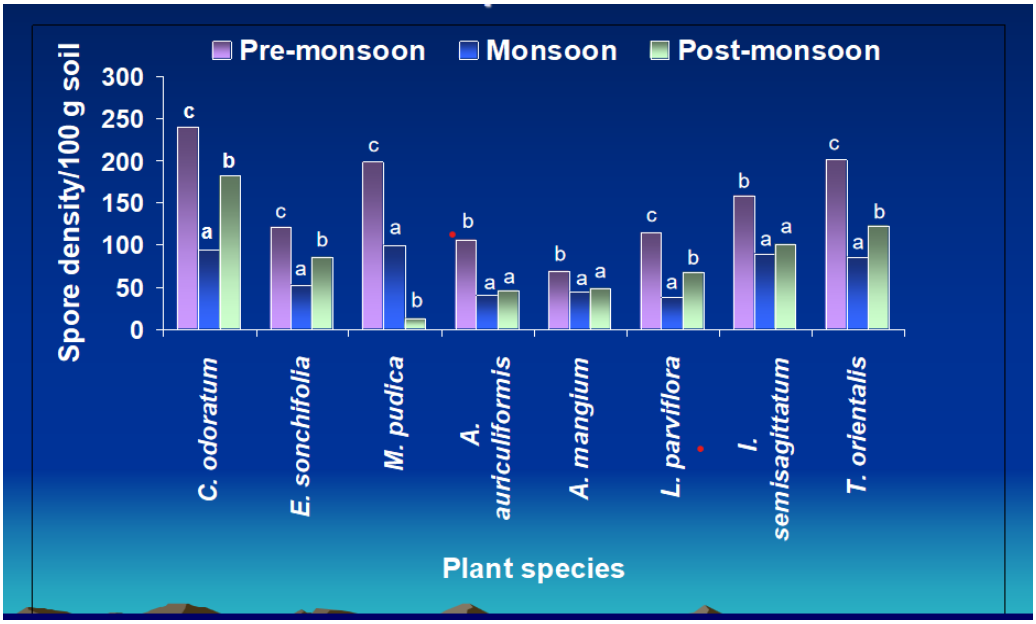

Figure 6: Seasonal variation in mean spore number. Columns of a season followed by same letter(s) is not significant according to DMRT (P<0.05). *C.=Chromolaena; E.=Emilia; M.=Mimosa; A.=Acacia; L.=Ludwigia; I.=Ischaemum; T.=Trema.*

Table 4: Three-way analysis of variance (ANOVA) of the data on spore numbers of eight plant species for three seasons over a year.

	d.f.	F ratio	Significance
Plant species (P)	7	79.18	**
Season (S)	2	200.37	**
P x S	14	3.23	**

Significant at: ** - P < 0.01; d.f., Degrees of freedom.

Relationship between spore number and AM colonization

Spore number and hyphal colonization were negatively correlated in *E. sonchifolia*, *A. mangium*, *I. semisagittatum* (P<0.01), and *M. pudica* (P<0.5). A highly significant (P<0.01, 0.000) negative correlation existed between spore number and arbuscular colonization in all the plant species. Vesicular colonization and spore numbers were negatively correlated to each other in *A. auriculiformis*, *I. semisagittatum*, *L. parviflora* (P<0.05), and *A. mangium* (P<0.01). Total root length colonization and spore number showed significant (P<0.01, 0.001, 0.000) negative correlation in *C. odoratum*, *M. pudica*, *A. auriculiformis*, *T. orientalis*, *A. mangium*, *E. sonchifolia*, *I. semisagittatum*, and *L. parviflora* at different levels of significance (**Table 5**).

Relationship between AM fungal colonization and edaphic factors

A highly significant (P< 0.05, 0.01, 0.001, 0.000) positive correlation existed between soil moisture and root length colonized by hyphae, arbuscules, vesicles, and total root length colonization in all the plant species (**Tables 6-9).**

Table 5: Correlation between arbuscular mycorrhizal (AM) fungal spore number and AM fungal structures.

Plant species	Hyphae	Arbuscules	Vesicles	Total colonization
C. odoratum	-0.377	-0.755****	0.281	-0.604**
E. sonchifolia	-0.678**	-0.806****	-0.250	-0.865****
M. pudica	-0.490*	-0.837****	0.423	-0.657**
A. auriculiformis	-0.361	-0.783****	-0.494*	-0.680**
A. mangium	-0.592**	-0.604**	-0.658**	-0.703***
L. parviflora	-0.414	-0.895****	-0.487*	-0.837****
I. semisagittatum	-0.675**	-0.811****	-0.589*	-0.840****
T. orientalis	-0.375	-0.845****	-0.253	-0.677**

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000
C.=Chromolaena, E.=Emilia, M.=Mimosa, A.=Acacia, L.=Ludwigia, I.=Ischaemum, T.=Trema.

Table 6: Correlation between root length colonized by hyphae and edaphic variables.

Plant species	SM (%)	pH	EC	N	P	K	Ca	OC (%)	OM (%)
C. odoratum	+0.520*	-0.390	-0.427	-0.057	-0.457	-0.304	-0.383	-0.085	-0.418
E. sonchifolia	+0.670**	-0.381	-0.512*	-0.204	-0.576*	-0.683**	-0.445	-0.175	-0.262
M. pudica	+0.588**	-0.141	-0.321	+0.588**	-0.462	-0.557*	-0.388	-0.529*	-0.329
A. auriculiformis	+0.547*	-0.387	-0.296	-0.010	-0.534*	-0.550*	-0.311	-0.300	-0.352
A. mangium	+0.852****	0.021	-0.486*	-0.756****	-0.734***	-0.802****	-0.654**	-0.745****	-0.712***
L. parviflora	+0.507*	-0.493*	-0.703***	0.115	-0.377	-0.336	-0.411	-0.078	-0.007
I. semisagittatum	+0.739****	0.031	-0.444	-0.597**	-0.700***	-0.630**	-0.656**	-0.545*	-0.208
T. orientalis	+0.492*	-0.194	-0.674***	-0.210	-0.375	-0.262	-0.558*	-0.318	-0.057

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000
SM- Soil moisture, EC-Electrical conductivity, OC- Organic carbon, OM- Organic matter
C.=Chromolaena, E.=Emilia, M.=Mimosa, A.=Acacia, L.=Ludwigia, I.=Ischaemum, T.=Trema.

Table 7: Correlation between arbuscular colonization and edaphic variables.

Plant species	SM (%)	pH	EC	N	P	K	Ca	OC (%)	OM (%)
C. odoratum	+0.834****	0.051	-0.581**	-0.063**	-0.734***	-0.810****	-0.522*	-0.553*	-0.486*
E. sonchifolia	+0.936****	-0.172	-0.608**	-0.591**	-0.889****	-0.961***	-0.661**	-0.628**	-0.609**
M. pudica	+0.908****	-0.056	-0.691**	-0.541*	-0.822****	-0.795****	-0.576*	-0.555*	-0.468
A. auriculiformis	+0.640**	0.393	-0.319	-0.718***	-0.691**	-0.785****	-0.237	-0.585**	-0.166
A. mangium	+0.765****	0.174	-0.500*	-0.585**	-0.627**	-0.655**	-0.490*	-0.626**	-0.233
L. parviflora	+0.931****	-0.023	-0.656**	-0.648**	-0.881****	-0.926****	-0.615**	-0.665**	-0.547*
I. semisagittatum	+0.640**	0.070	-0.483*	-0.699***	-0.647**	-0.661**	-0.402	-0.487*	-0.456
T. orientalis	+0.890****	0.035	-0.614**	-0.657**	-0.738****	-0.753****	-0.675**	-0.681**	-0.420

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000
SM- Soil moisture, EC-Electrical conductivity, OC- Organic carbon, OM- Organic matter
C.=Chromolaena, E.=Emilia, M.=Mimosa, A.=Acacia, L.=Ludwigia, I.=Ischaemum, T.=Trema.

Table 8: Correlation between root length colonized by vesicles and edaphic variables.

Plant species	SM (%)	pH	EC	N	P	K	Ca	OC (%)	OM (%)
C. odoratum	-0.171	0.421	0.329	-0.335	0.207	0.008	0.160	-0.114	-0.116
E. sonchifolia	-0.052	0.172	-0.210	-0.002	-0.048	-0.100	0.022	0.013	0.189
M. pudica	0.375	-0.256	0.055	+0.598**	0.401	+0.552*	0.160	+0.534*	0.309
A. auriculiformis	+0.780****	-0.453	-0.755**	-0.068	-0.665**	-0.648**	-0.568*	-0.307	-0.261
A. mangium	+0.646**	0.253	-0.255	-0.687**	-0.583*	-0.711***	-0.331	-0.734***	-0.304*
L. parviflora	+0.533*	-0.009	-0.126	-0.348	+0.548*	-0.674**	-0.287	-0.433	-0.125
I. semisagittatum	+0.535*	-0.022	-0.406	-0.473*	-0.586*	-0.534*	-0.354	-0.267	-0.240
T. orientalis	0.353	0.150	-0.116	-0.490*	-0.461	-0.349	-0.281	-0.447	-0.335

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000
SM- Soil moisture, EC-Electrical conductivity, OC- Organic carbon, OM- Organic matter
C.=Chromolaena, E.=Emilia, M.=Mimosa, A.=Acacia, L.=Ludwigia, I.=Ischaemum, T.=Trema.

Table 9: Correlation between total root length colonization and edaphic variables.

Plant species	SM (%)	pH	EC	N	P	K	Ca	OC (%)	OM (%)
C. odoratum	+0.7494****	-0.015	-0.481*	-0.558*	-0.630**	-0.708***	-0.461	-0.460	-0.562*
E. sonchifolia	+0.8356****	-0.275	-0.671**	-0.389	-0.792****	-0.798****	-0.593**	-0.462	-0.445
M. pudica	+0.781****	-0.300	-0.644***	-0.166	-0.661**	-0.605**	-0.539*	-0.398	-0.394
A. auriculiformis	+0.807****	-0.210	-0.592**	-0.308	-0.785	-0.813****	-0.467	-0.480	-0.333
A. mangium	+0.852*****	0.150	-0.475*	-0.784****	-0.751****	-0.837****	-0.569	-0.771****	-0.509*
L. parviflora	+0.881****	-0.233	-0.644**	-0.413*	-0.807****	-0.857****	-0.619**	-0.548*	-0.411
I. semisagittatum	+0.762****	0.001	-0.527*	-0.692**	+0.748****	-0.716***	-0.585**	-0.531*	-0.360
T. orientalis	+0.788****	-0.048	-0.646*	-0.569*	-0.682**	-0.605**	-0.693**	-0.621*	-0.373

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000
SM- Soil moisture, EC-Electrical conductivity, OC- Organic carbon, OM- Organic matter
C.=Chromolaena, E.=Emilia, M.=Mimosa, A.=Acacia, L.=Ludwigia, I.=Ischaemum, T.=Trema.

Table 10: Correlation between arbuscular mycorrhizal (AM) fungal spore number and edaphic variables.

Plant species	SM (%)	pH	EC	N	P	K	Ca	OC (%)	OM (%)
C. odoratum	-0.912****	0.265	+0.695***	0.395	+0.758****	+0.753****	+0.770****	+0.566*	0.427
E. sonchifolia	-0.894****	0.094	+0.708***	+0.525*	+0.683**	+0.699***	+0.735***	+0.621**	0.298
M. pudica	-0.868****	-0.140	+0.535*	+0.686**	+0.845****	+0.864****	+0.556*	+0.697***	0.347
A. auriculiformis	-0.817****	-0.162	+0.528*	+0.785****	+0.735***	+0.816****	+0.616**	+0.646**	0.365
A. mangium	-0.653**	-0.056	0.460	+0.597**	0.467	+0.628**	+0.575**	+0.491*	0.148
L. parviflora	-0.913****	0.202	+0.697***	+0.578**	+0.766****	+0.817****	+0.809****	+0.576*	+0.521*
I. semisagittatum	-0.735***	-0.035	0.465	+0.625**	+0.796****	+0.727***	0.454	+0.490*	0.384
T. orientalis	-0.897****	0.021	+0.497*	+0.551*	+0.859****	+0.854****	+0.612**	+0.682**	0.347

Probability levels: * - P< 0.05, ** - P< 0.01, *** - P< 0.001, **** - P< 0.000
SM- Soil moisture, EC-Electrical conductivity, OC- Organic carbon, OM- Organic matter
C.=Chromolaena, E.=Emilia, M.=Mimosa, A.=Acacia, L.=Ludwigia, I.=Ischaemum, T.=Trema.

Electrical Conductivity (EC), calcium, organic matter. Total soil N, P, K, and OC exhibited significant (P<0.05, 0.01, 0.001, 0.000) negative correlation with hyphal colonization in *A. mangium*. A highly significant (P<0.05, 0.01, 0.001) negative correlation existed between root length colonized by hyphae and OC, calcium, soil N, K, and P in *I. semisagittatum.* A significant (P<0.05, 0.001) negative correlation existed between pH, EC, and root colonized by hyphae in *L. parviflora*. In *E. sonchifolia,* hyphal colonization revealed a significant (P<0.05, 0.01) negative correlation with EC, P and K. Organic carbon and K showed negative (P<0.05) correlation with root length colonized by hyphae in *M. pudica* and in *T. orientalis* hyphal colonization exhibited significant (P<0.05, 0.01) negative correlation with calcium and EC (**Table 6**).

Root length colonized by arbuscules showed a significant (P<0.05, 0.01, 0.001, 0.000) negative correlation with soil N, P, K, and organic carbon in all the plant species. A significant (P<0.05, 0.01) negative correlation was observed between arbuscular colonization and OC in all the plant species. A significant (P<0.05, 0.01) negative correlation existed between EC and arbuscular colonization in all the plant species except *A. auriculiformis.* Similarly, arbuscular colonization exhibited a significant (P<0.05, 0.01) negative correlation with calcium in *C. odoratum, M. pudica, A. mangium, E. sonchifolia, L. parviflora* and *T. orientalis* (**Table 7).**

A highly significant (P<0.05, 0.01, 0.001) negative correlation existed between vesicular colonization and soil P and vesicular colonization and soil K in *I. semisagittatum, L. parviflora, T. orientalis,* and *A. auriculiformis.* A significant (P<0.05, 0.01, 0.000) positive correlation existed between vesicular colonization and soil moisture in *I. semisagittatum, L. parviflora, A. mangium,* and *A. auriculiformis*. Vesicular colonization and EC exhibited a significant (P<0.000) negative correlation in *A. auriculiformis.* A significant (P<0.05, 0.01) negative correlation was observed between soil N and vesicular colonization in *A. mangium, I. semisagittatum,* and *T. orientalis.* In comparison, it exhibited a significant (P<0.01) positive correlation in *M. pudica* **(Table 8)**.

Soil P, K, and EC exhibited significant (P<0.05, 0.01, 0.001, 0.000) negative correlation with total root length colonization in all the plant species except *I. semisagittatum* where P exhibited positive (P<0.000) correlation with total colonization. Soil organic matter and total root length colonization revealed significant (P<0.05) negative correlations in *C. odoratum* and *A. mangium*. Organic carbon and total root length colonization exhibited significant (P<0.05, 0.01, 0.000) negative correlation in *I. semisagittatum, L. parviflora, A. auriculiformis, T. orientalis,* and *A. mangium.* A significant (P< 0.01,0.001) negative correlation existed between calcium and total root length colonization in *M. pudica, A. auriculiformis, T. orientalis, I. semisagittatum, L. parviflora, and E. sonchifolia* **(Table 9)**.

Relationship between spore number and edaphic factors

Mean spore numbers exhibited significant (P< 0.01, 0.001, 0.000) negative correlation with soil moisture in all the plant species. Spore numbers showed a significant (P<0.05, 0.001,0.000) positive correlation with percent organic carbon, EC, total soil P, K, and calcium in *C. odoratum.* While EC, N, P, K, and OC showed a significant (P<0.05, 0.01, 0.001) positive correlation with spore number in *M. pudica, A. auriculiformis, T. orientalis, L. parviflora, E.sonchifolia,* and *A. mangium*. In *I. semisagittatum,* a significant (P<0.05, 0.01, 0.001, 0.000) positive correlation was noted between spore number with OC, total soil N, K, and P. Spore numbers exhibited significant (P<0.05) positive correlation with organic matter in *L. parviflora* **(Table 10)**.

Arbuscular mycorrhizal fungal spores

In the present study, 40 AM fungal species belonging to 13 Glomeromycota genera *viz.*, *Acaulospora* (9), *Archaeospora* (1), *Cetraspora* (1), C*laroideoglomus* (1), *Dentiscutata* (2), *Funneliformis* (2), *Gigaspora* (4), *Glomus* (11), *Paraglomus* (2), *Racocetra* (3), *Rhizophagus* (2), S*cutellospora* (1) and *Septoglomus* (1), were reported of which ten were common to all the three seasons. The genus *Glomus* was dominant in the pre-monsoon season, *Acaulospora* in the monsoon season, and *Gigaspora* in the post-monsoon season (**Table 11 and 12**).

DISCUSSION

The result of the present study indicated that environmental factors, host species, and soil type strongly influenced spore production and AM fungal colonization. Similar results have been recorded from earlier studies (Muthukumar *et al*., 1998; Sivakumar, 2013; Vieira Junior *et al.*, 2020).

The present study revealed that among the climatic factors, rainfall and Relative Humidity (RH) played an important role in root colonization and spore formation. The results agree with Gaur and Kaushik (2012), who have reported reduced spore count under higher humidity and moisture content on seasonal variation in AM fungi associated with medicinal plants of the Himalayan region of India. Michelini *et al*. (1993), in their studies on *Citrus*, related the AM fungal colonization in the roots to high rainfall.

Table 11: Season wise occurrence of AM fungal genera.

	Number of AM fungal species		
AM fungal genera	**Pre-monsoon**	**Monsoon**	**Post-monsoon**
Acaulospora	04	08	03
Archaeospora	01	01	-
Cetraspora	01	01	01
Claroideoglomus	01	01	-
Dentiscutata	01	01	02
Funneliformis	02	02	01
Gigaspora	03	01	04
Glomus	10	05	02
Paraglomus	02	-	01
Racocetra	03	02	03
Rhizophagus	02	01	01
*Scutellospor*a	-	-	01
Septoglomus	01	-	01
Total AM fungal species	31	22	19

Table 12: Seasonal variation in frequency of occurrence of AM fungal species in selected host plants from Codli iron ore mine wasteland.

AM fungal species	**Frequency (%)**		
	Pre-monsoon	**Monsoon**	**Post-monsoon**
Acaulospora (9)			
Acaulospora delicata Walker, Pfeiffer and Bloss	-	25	-
Acaulospora foveata Trappe and Janos	25	-	-
Acaulospora laevis Gerd. and Trappe	-	25	12
Acaulospora longula Spain and Schenck	-	12.5	-
Acaulospora mellea Spain and Schenck	-	12.5	-
Acaulospora morrowiae Spain and Schenck	-	12.5	-
Acaulospora rehmii Sieverding and Toro	25	12.5	-
Acaulospora scrobiculata Trappe	100	60	37
Acaulospora spinosa Walker and Trappe	50	62.5	50
Archaeospora (1)			
Archaeospora undulata Sieverd., G.A. Silva, B.T. Goto and Oehl	12	12.5	-
Cetraspora (1)			
C. pellucida (Nicol. and Schenck) Oehl, Souza and Sieverd	62	75	37
Claroideoglomus (1)			
C. claroideum (N.C. Schenck and G.S. Sm.) C. Walker and A. Schüßler	12	25	-
Dentiscutata (2)			
D. nigra (Redhead) Sieverd, Souza and Oehl	-	-	12
D. reticulata (Koske, Miller and Walker) Sieverd., Souza and Oehl	25	25	50

AM fungal species	Frequency (%)		
Funneliformis (2)			
F. mosseae (Nicol. and Gerd.) Walker and Schuessler	12	12.5	-
F. geosporus (Nicol. and Gerd.) Walker and Schuessler	62	37.5	37
Gigaspora (4)			
G. albida Schenck and Smith	12	-	12
G. decipiens Hall and Abbott	-	-	12
G. margarita Becker and Hall	75	62.5	75
G. rosea Nicolson and Schenck	12	-	12
Glomus (11)			
G. clavispora (Trappe) Almeida and Schenck	-	12.5	-
G. coremioides (Berk. and Bromme) Morton and Redecker	12	-	-
G. formosanum Wu and Chen	12	-	-
G. globiferum Koske and Walker	37	25	-
G. hoi Berch and Trappe	12	-	-
G. macrocarpum Tul. and Tul.	75	75	75
G. magnicaule Hall	12	-	-
G. multicaule Gerd. and Bakshi	12	-	-
G. rubiformis (Gerd. and Trappe) Almeida and Schenck	25	-	25
G. sinuosum (Gerd. and Bakshi) Almeida and Schenck	12	12.5	-
G. taiwanensis (Wu and Chen) Almeida and Schenck	37	37.5	-
Paraglomus (2)			
P. albidum Oehl, G.A. Silva and Sieverd	12	-	12
P. occultum (Walker) Morton and Redecker	12	-	-
Racocetra (3)			
R. gregaria (Schenck and Nicol.) Oehl, Souza and Sieverd	75	62.5	100
*R. persica (*Koske and Walker) Oehl, Souza and Sieverd	37	-	12
R. weresubiae (Koske and Walker) Oehl, Souza and Sieverd	25	12.5	25
Rhizophagus (2)			
R. aggregatus (N.C. Schenck and G.S. Sm.) C. Walker	12	-	-
R.fasciculatus (Thaxter) Walker & Schuessler	62	50	50
Scutellospora (1)			
S. calospora (Nicol. and Gerd.) Walker and Sanders	-	-	12
Septoglomus (1)			
S. constrictum (Trappe) Sieverd., G.A. Silva and Oehl	37	-	25

The present investigation observed that AM colonization was reduced during the dry season (pre-monsoon). Results reported in the present study agree with Halder *et al*. (2015), who found a reduction in the percentage of AM colonization in the roots of medicinal plant species during the dry season in Chittagong BCSIR forest. Thus, various AM fungi may respond differently to temperature changes. Soil temperature might alter the physiology of mycorrhizal symbiosis by influencing root morphology, nutrition, and growth. The influence of temperature on AM fungal colonization and sporulation has been reported in earlier studies (Allen, 1983; Udaiyan *et al*., 1996).

Root colonization and spore density results indicated that the extent of mycorrhizal colonization (hyphal, arbuscular, vesicular, and total) and spore density varied significantly in pre-monsoon, monsoon, and post-monsoon seasons. Similarly, Muthukumar *et al.* (1998) reported within-season variations of AM colonization and spore abundance in the rhizosphere soils in relation to edaphic factors in eight wild legumes. In the present study, the colonization levels in most plant species varied when the plants were considered individually, indicating that different AM fungal species colonize different plant species. The structure and intensity of AM colonization vary within plant populations and depend on factors such as type and spatial availability of inoculum, season, stage of plant development, susceptibility to inoculation, and plant nutritional status. Seasonal variations in spore populations in different plant species have been reported earlier (Halder *et al*., 2015).

In the present study, soil moisture positively correlated with mycorrhizal colonization and negatively correlated with spore density (Halder *et al*., 2015; Santos *et al*., 2007). The occurrence of maximum arbuscular colonization during monsoon suggests that low temperatures may favour its formation. During this period, the fungus is actively involved in nutrient transfer. The production of vesicles increased at the end of the maximum root growth, the formation of new arbuscules ceased, and the older ones disintegrated. As a result, the arbuscular colonization was minimal in pre-monsoon, during which vesicles became more prominent in the cortex of older roots. The high percentage of AM colonization correlates with the active growth of the host plants. Optimum moisture for plant growth may favour mycorrhizal formation due to the availability of new roots, which in turn stimulates AM fungal spore germination. As a result, maximum colonization has been recorded during monsoon. The changes observed in the colonization pattern with soil moisture agree with Halder *et al.* (2015), who have shown that the proportion of root length colonized by hyphae and arbuscules could fluctuate with soil moisture.

Spore density was maximum in all the plants during pre-monsoon and declined in monsoon and post-monsoon. Vieira Junior *et al*. (2020), in their studies on seasonal variation in the mycorrhizal community of different cerrado phytophysiomies, revealed that the mycorrhizal population is influenced by the season. They reported increased mycorrhizal activity in dry seasons compared to the rainy seasons. Sivakumar (2013) reported increased spore number during the dry season and attributed the variation in AM fungal population to soil edaphic and climatic factors. Earlier studies have revealed that low temperature reduces AM formation (Gavito and Azcón-Aguilar, 2012; Santos *et al*., 2007).

Soil P was negatively correlated to mycorrhizal colonization. The low soil P is known to increase root colonization by AM fungi (Mosse and Hayman, 1971). Muthukumar *et al.* (1994, 1998) and Udaiyan *et al*. (1996) stated that soil P can reduce AM formation, and the inhibition may be due to the direct effect on external hyphal growth. While Sanders (1975) suggested that inhibition of AM fungal colonization may be indirectly associated with host P status. Ratnayake *et al*. (1978) and Graham *et al*. (1981) have suggested that AM fungi inhibit root colonization at high P levels because of decreased root exudation. In the present study, spore density was positively correlated to soil P in all the plant species. Similar results were reported in an earlier study by Udaiyan *et al*. (1996).

Soil N was negatively correlated to mycorrhizal colonization and positively correlated to spore number. These results conform with Redhead (1975), who found that N strongly affects mycorrhizal colonization in *Khaya grandifolia*, whereas it enhanced the number of spores in the rhizosphere. Similarly, Thomazini (1974) found that Brazilian soils low in Ca, P, S, N, and Zn were associated with good AM colonization. The present study observed no marked effect of pH on root colonization and spore number. These results agree with the earlier findings of Johnson *et al*. (1991) and Khalil and Loynachan (1994), who found no relation between soil pH and either root colonization or spore number. However, a negative correlation between AM colonization and OC and organic matter was recorded in the present study. These results agree with Nicolson (1960), who has reported a decrease in mycorrhizal colonization with increasing organic matter content. However, our results contradict the observations made by Sivakumar (2013), who reported a positive correlation between pH, AM fungal spore density, and root colonization and a negative correlation between EC, nitrogen, and P.

In the present study, K had a negative effect on root colonization. These results are in accordance with Melo *et al*. (2018). However, the spore number was positively correlated to K. Calcium exhibited a negative correlation with AM fungal colonization and a positive correlation with AM fungal spore numbers, which suggests that calcium fluctuation in rhizosphere governs the vegetation and reproductive phases of AM fungi in the mine wastelands. Anderson *et al.* (1984) reported a negative correlation between calcium and plant root length colonization.

The total number of AM fungal spores recorded was maximum in pre-monsoon. *Glomus* was the most dominating genera during this period, followed by *Acaulospora* and *Scutellospora*. Whereas, during monsoon, *Acaulospora* were more in number, followed by *Glomus* and *Scutellospora*. During post-monsoon, *Gigaspora* was the most dominant genera. In the present study, a seasonal pattern of sporulation has been reported. At the same time, some species were seen occurring around the year, which suggests that they vary in their response to changes in the abiotic environment (Soka and Ritchie, 2014; Bouamri *et al*.,2014).

In the present study, a large number of *Acaulospora* species were reported during monsoon, while *Gigaspora and Scutellospora* were more in pre-monsoon and post-monsoon. Thus, the increased soil temperature during pre-monsoon and post-monsoon may favour the formation of *Gigaspora* and *Scutellospora*. Similarly, seasonality in the development of AM fungi includes the period when the spores are likely to be released in the soil, and the presence of some immature spores suggests some production of spores throughout the year (Hayman, 1970).

This study indicates that seasonal variations remarkably influence the occurrence and distribution of AM fungal spore number and root colonization, the host species, and the soil type. This study would enable the selection of functionally complementary species or their combinations, which could be of considerable importance for the success of mine wasteland stabilization programmes, indicating a good scope for further intensive work throwing more light on the ecology of AM fungi on mine wastelands.

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