Seasonal status of Mycorrhizal fungi of silver cock's comb (*Celosia argentea* L.)

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Abstract: **The present study determined the seasonal variation of AM fungi in *Celosia argentea* L. The sample of roots and rhizosphere soil of *C. argentea* was studied from 10 different study sites. The percentage, types of root colonization and AMF spore density from three different seasons were studied.The winter season had the highest percentage of root colonization and spore density, followed by the summer season and the monsoon season had the lowest. The maximum percentage of root colonization in Naldurg (64±4.0) in the winter season and minimum in the monsoon (4.0) from Murtha Horti and Nilegaon. Vesicular, arbuscular and hyphal types of root colonization were recorded in three seasons. The highest percentage of root length colonization was recorded in the winter season (51.2 ±21.95) from Naldurg and the lowest in summer (2.0)from Kesarjawalga and Hangarga. The appearance of AM fungal spore density varied from site to site and season to season. In winter, maximum spore density was recorded in Hangarga (377±19.30/100g soil) and minimum in Gujnur and Chikundra (85.5) in monsoon season. Four different AMF genera and 12 species were recorded, i.e., *Acaulospora*, *Glomus*,*Gigaspora* and *Sclerocystis* from three different seasons. *Glomus* sp. was found to be dominant over others. *Glomus citricola* had the highest relative abundance (16.66%) as well as frequency of occurrence (75%) and *Sclerosystis rubiformis* and *Sclerosystis sinuosa* have the lowest (3.7%). The present study shows that the seasonal variation controls the % infection rate and % spore population. Physco-chemical parameter of soil affects the infection of the AM fungi in the host plant. The winter season soil has the most favourable and rich in all physio-chemical parameters followed by the summer and rainy seasons show the less rich soil nutrients among the three.**

Keywords: **Rhizosphere, Study sites, AMF status, seasonal variations, *Celosia argentea* L.**

INTRODUCTION

Celosia argentea is an herbaceous plant that belongs to the family amaranthaceae. It is usually known as the plumed cockscomb or silver cockscomb. Its leaves are eaten and used as leafy vegetables (Uusiku et. al., 2010). It is a short-day plant having alternate, entire, rarely lobed leaves. The plant is an erect, simple branched, smooth annual herb with a height of 0.5 to 1.5 m. plants with spikes of pinkish or white flowers ranging in size from 8 to 12 mm. *C. argentea* flowers produce a large number of seeds that range in size from 1 mm to 1 mm and are typically black in colour (Ron et. al., 1995).

The leaves are rich in protein and vitamins, and the stems are applied as dressings for infected sores, wounds, and skin eruptions, as well as used to relieve gastrointestinal disorders and as an antipyretic. Seeds, when in decoction or finely powdered, are considered antidiarrheal or aphrodisiac. The entire plant is used as a snake poison antidote, while the root is used to treat abdominal colic and gonorrhoea (Priya et. al., 2008). Shwetha et.al. (2010) recorded the AM fungal infection in *C. argentea*.

Farmers use massive amounts of chemical fertilisers without realising the impact on soil health. This leads to the accumulation of toxic salts in the soil and the resultant arrested plant growth.

An arbuscular mycorrhizal fungus belongs to the phylum Glomeromycota. It is a type of endotrophic fungi present in nearly all terrestrial ecosystems (Wu et. al., 2016). It is mutualistic association occurs when fungi provide minerals and water to the host plant and the plant provides photosynthetic products to the fungi in the form of sugar; both are mutually beneficial (Smith and Read, 2008). The fungal hyphae develop inside the host plant's cortex cells, known as endomycorrhiza and around the cortex cell, known as ectomycorrhiza (Gutierrez et. al., 2003). An about 90% of the plant species of the terrestrial ecosystem were associated with AM fungi, apart from Brassicaceae and a few other families (Liu et. al., 2007). Mycorrhizae afford different benefits to plants and the environment. It enhances the photosynthesis rate, increases the plant's establishment and survival, increases the yield and crop quality, develops drought tolerance, improves

flowering and fruiting, reduces the use of chemical fertilizers, solubilizes phosphorus, increases tolerance to soil salinity, and reduces disease occurrence, especially soil-borne pathogens (Ebrahim Sedaghati, 2014). Spore quantification is an important principle for determining the association of AM fungi in soil because spores are resistant to adverse conditions (Harley and Smith, 1983).

Considering the beneficial effect of AMF on other crops, more consideration should be paid to combining appropriate mycorrhizal fungi inoculum to get a good yield of crop plants. Hence present study enlists AM fungal association with the *C. argentea*, studied the diversity of AMF spore and quantifies the spore density.

MATERIAL AND METHODS

Study area- The Osmanabad district is situated in the southern part of the Marathwada region between latitude 17.35 to 18.40 degrees north and latitude 75.16 to 76.40 degrees east. An investigation was carried out from 10 different study sites i.e., Naldurg, Horti, Murta, Chikundra, Kesarjalwga, Nilegaon, Khudawadi, Gujnur, Hangarga, Osmanabad etc. Temperature ranges from 10.1-431^{0}C and average precipitation per year is 760 mm.

Collection of Rhizosphere sample - Rhizosphere soil and root samples of *C. argentea* were collected from the 10 different study sites of the Osmanabad district. The soil samples were collected from underground root's surface and surroundings. A sterilized scalpel was used to transfer rhizosphere soil into sterilized zip-lock polythene bags separately. The secondary root of *C. argentea* was washed with clean water removed soil debris and stored in FAA solution (Formalin-Acetic Alcohol in a separate sterilized glass bottle.

Physicochemical parameter of soil- The soil pH is measured by a pH meter using 1:5 soil and water suspension. Ec is calculated by using a conductivity meter which runs the concentration Of salts in the soil and measured in 1:5 soil water suspension. Organic carbon was assessed by oxidizing organic carbon with potassium dichromate and sulphuric acid method given by (Walkely and Black 1934). Nitrogen was measured by using the Kjeldhal tube method (Subbiah and Asija, 1956). The phosphorus content was determined by using the spectrophotometer given by the Olsens method (Olsen et. al., 1954; Bray & Kurtz 1945). The potassium was assessed by Flame photometer using the Ammonium acetate method (Hanway and Heidel 1952). The EDTA titration was used to estmation of Calcium and Magnesium cations (GOI, 2011b). the estimation of manganese, Copper and Zinc and Ferrous was carried out by using acid digestion of soil (Jackson, 1967).

Assessment of arbuscular mycorrhizal status- Primary and secondary fine roots were selected and washed 3–4 times to remove the FAA from the root. 20-30 root segments were cut into 2-3 cm lengths and boiled in 10% KOH. Boiled root sample wash with sterilized distilled water until the brown colour changes to colourless. The root was immersed in 5% HCL for 3–4 minutes to improve staining ability. Acidified root was washed with sterile distilled water for 4-5 times and stained with 0.05% trypan blue stain overnight (12 hours). The root was placed on a clean slide after the excess stain was removed. Vesicular, arbuscular and hyphal AM fungal colonization was observed and the results were recorded and photographed with a Sony digital camera (DSC-W310/BC E37) (Phillips et. al., 1970). The percentage of root colonization was assessed by using the formula (Giovannetti and Mosse, 1980).

$$\text{Root colonization (\%)} = \frac{\text{Number of colonized segments}}{\text{Total no. of colonized segments studied}} \text{X100}$$

Isolation and quantification of AMF spores: Isolation of spores from each soil sample was done by wet sieving and decanting method (Gerdmann and Nicolson, 1963). The100 gm of soil sample was dissolved in 1000 ml of tap water and this suspension was passed through 355 µm 250µm, 150µm, 125µm and 63 µm sieves. Spore was collected by using Whatman filter paper. Each filter paper was spread onto a glass plate and scanned under a stereozoom microscope. Intact and shiny spore was counted and picked up using a wet needle and mounted in polyvinyl alcohol lactophenol (PVLG) on a glass slide and identified under a compound microscope and photographed Sony digital camera (DSC-W310/BC E37).

Identification of spore: Identification was based on spore morphology and subcellular characters (Schenck and Perez, 1990). Identification of AM fungal spores was carried out by using the INVAM International Collection of Vesicular Arbuscular

Mycorrhizal morphtaxonomic criteria and mycorrhizal manuals (Schenck et. al., 1998; Rodrigues et. al., 2009). The voucher specimens of AM fungi were deposited at the Department of Botany, Arts, Scienc e and Commerce College Naldurg, Dist. Os manabad and Department of Botany, Dr. Babasaheb Ambedkar Marathwada University in Aurangabad, Maharashtra, India.

Relative abundance and frequency of occurrence of AM fungi – Spore population was recorded as well as relative abundance and frequency of occurrence also calculated by using following formula (Giovannetti and Mosse, 1980).

$$\text{RA (\%)} = \frac{\text{Number of AM fungal spore of a particular species}}{\text{Total no. of AM fungal spore in species}} \text{X100}$$

$$\text{F (\%)} = \frac{\text{Number of soil sample possessing spores of a particular AM species}}{\text{Total no. of soil sample analyzed}} \text{X100}$$

Statistical analysis: All data were statistically analyzed and the importance of variances was determined by using a book (Mungikar, 1990).

EXPERIMENTAL RESULTS

Physicochemical parameter: The pH, EC, OC, N, P K, Na Caco3, Fe, Mn, Zn, Cu, S, and B parameters were studied from the rhizosphere of different seasons. The parameters were studied by comparing their standard values (Table 1). The pH of the soil was neutral in all three seasons. electric conductivity is moderate in the winter season (0.52 ds/m^2) as compare to other. The organic carbon (OC) rhizosphere shows the highest appearance (0.76%) and lowest (0.23%) in rainy season. Nitrogen has the highest in winter (183kg/ha) and lowest in rainy(55.43kg/ha) seasons. Phosphorus shows the highest in the winter season (19.89 kg/ha) and moderate in other two seasons.

Potassium occurs maximum in the three seasons (14 21kg/ha), (456kg/ha) and (689kg/ha) respectively. Sodium content in the soil during winter seasons was highest (2.14mmol/L) and lowest summer (0.40mmol/L). Calcium carbonate ($CaCo_3$) was moderately present in all three seasons (5.94),(4.86) and (2.7) respectively. Iron content lowest in all three season. The manganese (Mn) content in all three seasons is also the lowest . The zinc (Zn) highest in all the seasons.(Copper (Cu) content was lowest in all three seasons. Sulfur and Boron were absent in all three seasons.

Root colonization: A root sample from ten different locations and three different seasons was analysed Vesicular, arbuscular and hyphal root colonization was recorded. the percentage (%) of root colonization and the percentage (%) of root length colonization results was recorded (table no.2). In the winter season, the maximum % of root colonization was recorded in Naldurg (64±4.0) and the minimum in Gujnur (4.4). During the summer season, Osmanabad had the highest percentage of root colonization (12+7.05) and Khudawadi, Gujnur, and Chikundra had the lowest (4.0). During the monsoon, Hangarga has the highest percentage of root colonization (8.0) and Murta, Horti, and Nilegaon have the lowest (4.0) Among the three seasons winter season had the highest percentage of root colonization followed by the summer season and the monsoon season had the lowest (Fig 1).

Root length colonization: In the winter season, the maximum % of root length colonization was recorded, Naldurg (51.2±21.95) had the highest percentage of root length colonization and Gujnur (2) had the lowest. Maximum root length colonization in the summer season was recorded in Osmanabad (8±1.50) and minimum in Hangarga khudawadi, Gujnur and Chikundra (3). During the monsoon season, Naldurg had the highest percentage of root length colonization (6.0± 3.12) while Kesarjawalga lowest (2).

Spore density :The appearance of AM fungal spore density varied from site to site and season to season. In winter, maximum spore density was recorded in Hangarga (377±19.30/100g soil) and minimum in Nilegaon (177±4.32/100g). In the summer season, maximum spore density appears in Naldurg (233±6.90/100g soil) and minimum in Horti (130±0.40/100g soil). Hangarga had the highest spore density (229 ± 10.10 / 100 g) during the monsoon season, while Gujnur and Chikundra had the lowest (85.5 ± 0.0 /100 g) (Table no.3). Four different AMF genera and 12 species were recorded, i.e., *Acaulospora dilatata, A. gerdemanii, Glomus fasciculatum, G. citricola, G. clarum, G. geosporum, G. macroagregatum, G. radiatum, Gigaspora gigantean Gi. margarita, Sclerosystis rubiformis* and *S. sinuosa* among them *G. citricola* had the highest relative abundance (16.66%) as well as frequency of occurrence (75%) and *Sclerosystis rubiformis and S. sinuosa* have the lowest (3.7) and (16) respectively. *Glomus* sp. was found to be dominant over others (Fig.2).

DISCUSSION

Brundrett (2017) previously stated that plants from the Amaranthaceae, caryophylaceae, chenopodiaceae, cyperaceae, juncaceae, urticaceae, poaceae, polypodiales, proteaceae, and Brassicaceae families had rarely or never been associated with AMF. Bhale (2018) earlier reported AMF root colonization in the 12 species belonging to 8 families and 11 genera in rabi and 14 weed species belonging to 9 families and 13 genera in kharib season from the agricultural field. The highest colonization was recorded in *cassia tora* (85.1%) and C. *argentea* (81.26%) lowest in *Cyprus rotundus* (50%). Muthukumar and Udaiyan, (2000) previously reported AM fungal root colonization and spore density in *Achyranthes aspera* belongs to the family amranthaceae, from the Western Ghats region of southern India. The highest spore density was (11.7±64.13) with primarily hyphal, vesicular, and arbuscular association. Shwetha and Lakshman, (2010) previously reported that arbuscular mycorrhizal association was found in twenty-three plant species, mostly from the amranthaceae family, among *A. polygamus, A. caudatus, A. triandra,* and *C. argentea* having the arbuscules vesicles, and coiled hyphae is component of AM fungi. The maximum number of vesicles ranges from (11.50%) to (18.48%). Study revealed the thirty-five indigenous AM fungal spores while *Glomus* had the most records, while *Scutellospora* had the fewest similar to our finding.

The soil parameter plays a crucial role in the result of mycorrhizal infection. It is a combination of plant habits, light incidence, temperature, climatic characteristics, and soil moisture that make significant changes in the yield of crop plants (Zangaro et al., 2013).

Sreevani and Reddy (2004) observed the relation between soil and AMF fungi and displayed that neutral to slightly alkaline (pH 7-8) soil favours a greater number of AMF propagules while mycorrhizal fungal development did not favour higher pH i.e. 8.0 of the soil which is similar to our report. Tanvi et. al. (2019) discovered AMF root colonization in plant species from 36 families found in mine dumps in Goa. *A. sessilis, C. argentea, and A. viridis,* all members of the amaranthaceae family, were also studied, and moderate root colonization and spore density were observed. Kumar et. al. (2003) previously studied seventy-nine plant species belonging to 30 families growing at coal mine dumps of various ages. *C. argentea* having least root colonization with globular and sub globular vesicle. Mycorrhiza improves the phosphatase uptake capac ity as well as increases soil enzyme activities. Most studies have investigated earlier P uptake but mycor rhizae have been associated in the uptake also othe r essential nutrients by exploring its hyphae into the soil zone (Mar Vazquez et. al., 2000). Mycorrhiza plays an important role in improving plant growth b y various mechanisms. It plays a key role, particular ly under stressful environmental conditions. The co mbined application of mycorrhizae could be a signif icant approach to sustainable agriculture (Najafi et. al., 2012). AMF colonization enhances the photosynthesis and biomass accumulation in plants and also promotes the synthesis of chlorophyll and carotenoid, increases the root absorption area root activity, and strengthens the absorption and transport of water and other nutrients and mineral elements such as P, K, Mg, and Mn (Graham, 2000). The present study revealed the rhizosphere of *C. argentea* had diverse AM fungal spores, and root colonization was controlled by seasonal variation.

CONCLUSION

The present study confirms the diverse occurrence of AM fungi in *C. argentea* from 10 different study sites of the Osmanabad district. The mycorrhizal fungi have a major impact on host plants even under any environmental condition. Vesicular, arbuscular and hyphal root colonization and four different AMF genera and 12 species were recorded, i.e., *Acaulospora*, *Glomus*, *Gigaspora* and *Sclerocystis* from three different seasons. *Glomus* species were most visible and *G. citricola* had the highest relative abundance and frequency of occurrence.

Table-1. Physicochemical parameters of rhizosphere soil of amaranthus species.

Sr.no.	Parameters	Standered values	Summer	Monsoon	Winter
1.	pH	6.5-7.5	8.11	8.26	8.16
2.	EC(dS/m)	Less than 1.0	0.18	0.31	0.52
3.	OC (%)	0.41 to 0.60	0.57	0.23	0.76

4.	N (kg/ha)	161 to 320	139	55.43	183
5.	P (kg/ha)	31 to 50	18	20.00	19.89
6.	K(kg/ha)	181 to 240	456	689	1421
7.	Na(meq/L)	65 to 80	0.40	1.26	2.14
8.	Caco3	10 to 15	10.26	4.86	5.94
9.	Fe(ppm)	5 to 15	0.77	0.39	0.59
10.	Mn(ppm)	1.0 to 5.0	0.53	0.42	0.37
11.	Zn(ppm)	2.5 to 5.0	0.38	0.33	0.43
12.	Cu(ppm)	2.0 to 5.0	0.10	0.11	0.20
13.	S	0.2 to 0.5	-	-	-
14.	B(ppm)	0.2 to 2.0	-	-	-

Table 2. Showing the status of percentage (%) of AMF root, root length and type of root colonization from three different seasons (n*=25).

Sr.no	Study site (Location)	(%) Root colonization			(%) -Root length colonization			Type of colonization		
		Summer	Monsoon	Winter	Summer	Monsoon	Winter	Summer	Monsoon	Winter
1.	Naldurg	8.0±4.0.	4.4±0.0	64±4.0.	4.0±0.0	6.0±3.12	51.2±21.95	HV	H	H
2.	Murta	8.0±4.0.	4.0±0.0	52±0.33	4.0±0.0	4.0±0.0	47.2±11.79	VH	VH	VH
3.	Horti	8.0±4.0	4.0±0.0	52±4.53	4.0±0.0	4.0±0.0	47.2±11.79	HVA	H	H
4.	Osmanabad	12±7.05	4.4±0.0	56±7.12	8.0±1.50	4.0±0.0	48.0±7.40	HV	H	H
5.	Nilegaon	8.0±0.0	4.0±0.0	56±7.12	4.0±0.0	4.0±0.0	48.0±7.40	H	H	H
6.	Kesarjawalga	8.0±0.0	4.4 ±0.0	33±4.32	4.0±0.0	2.0±0.0	31.0±1.32	HV	H	VH
7.	Hangarga	8.0±4.0	8.0±0.0	44±4.32	3.0±0.0	3.0±0.0	28.0±9.50	HV	H	VH
8.	Khudawadi	4.4±0.0	4.4±0.0	24±4.32	3.0±0.0	4.0±0.0	25.0±4.53	HV	H	VH
9.	Gujnur	4.4±0.0	4.4±0.0	8.0±0.0.	3.0±0.0	4.0±0.0	2.0±0.0	V	H	VH
10.	Chikundra	4.4±0.0	4.4±0.0	12.02±0.0.	3.0±0.0	4.0±0.0	3.0±0.0	V	H	VH

Legends:*-Number of Root segments, RC- root colonization, RLC- Root length colonization, H- hyphal, V-vesicle, A- Arbuscule, S-Summer, M-Monsoon, W-Winter Values are means of three replications, standard error (±).

Table 3- Showing AM fugal spore population from rhizosphere of *C. argentea* from different study site.

Sr.no.	Study site (Location)	Spore numbers /100g soil		
		Summer	Monsoon	Winter
1.	Naldurg	233±6.90	130±1.25	282±11.42
2.	Murta	229± 4.63	177±5.23	233±21.42
3.	Horti	130± 0.40	185±4.53	233±21.42
4.	Osmanabad	229± 4.35	130±1.32	243±33.21
5.	Nilegaon	177±5.50	130±1.32	177±4.32
6.	Kesarjawalga	185±5.50	185±3.01	297±10.02
7.	Hangarga	229±7.50	229±10.10	377±19.30
8.	Khudawadi	189±10.02	189±4.70	243±33.21
9.	Gujnur	173±6.02	85.5±0.0	233±21.42
10.	Chikundra	173±6.02	85.5±0.0	243+33.21

Values are means of three replications, standard error (±).

Table 4-Showing distribution of species of AM fungal spores in different study site, (%) relative abundance and (%) frequency of Osmanabad District.

Sr.no.	Species of AM fungi	Study site										%RA	% F
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10		
1.	*Acaulospora dilatata* J.B.Morton	+	-	+	-	+	+	+	-	-	-	9.25	41.66
2.	*A. gerdemanii* Nicoloson and Schenck.	+	+	-	+	+	+	+	-	-	-	11.11	50
3.	*Glomus fasciculatum* Gerdemann and Trappe emend Walker and koske	+	+	+	+	+	+	-	+	+	-	14.81	66.66
4.	*G. citricola* Tang and Zang.	+	+	+	+	+	-	+	+	+	+	16.66	75
5.	*G. clarum* Nicoloson and Schenck.	+	+	-	+	-	-	-	-	-	-	5.5	25
6.	*G. geosporum* (Nicoloson and Gerdemann) Walker.	+	+	-	+	-	-	-	-	-	-	5.5	25
7.	*G. macroagregatum* N.C.Schenck &G.S. Smith.	+	+	+	-	+	+	-	-	-	-	9.25	41.66
8.	*G. radiatum* (Thaxt.)Trappe & Gerd.	+	+	-	+	-	-	-	-	-	-	5.5	25
9.	*Gigaspora margarita* Becker and Hall.	-	+	+	+	+	+	-	_	_	-	9.25	41.66
10.	*Gigaspora gigantean* (Nicoloson and Gerdemann) Gerdemann and Trappe.	-	-	+	+	+	+	+	-	-	-	9.25	41.66
11.	*Sclerosystis rubiformis* Gerdemann &Trappe.	+	-	-	-	-	-	-	+	-	-	3.7	16
12.	*S. sinuosa* Gerdemann and Bakshi.	+	-	-	-	-	-	-	-	+	-	3.7	16

Legends:+: present - : absent S1-Naldurg S2- Murta S3- Horti S4- Osmanabad S5- Nilegaon S6- Kesarjawalga S7- Hangarga S8- Khudawadi S9- Gujnur S10- Chikundra.%RA- relative abundance, %F- Frequency of occurrence.

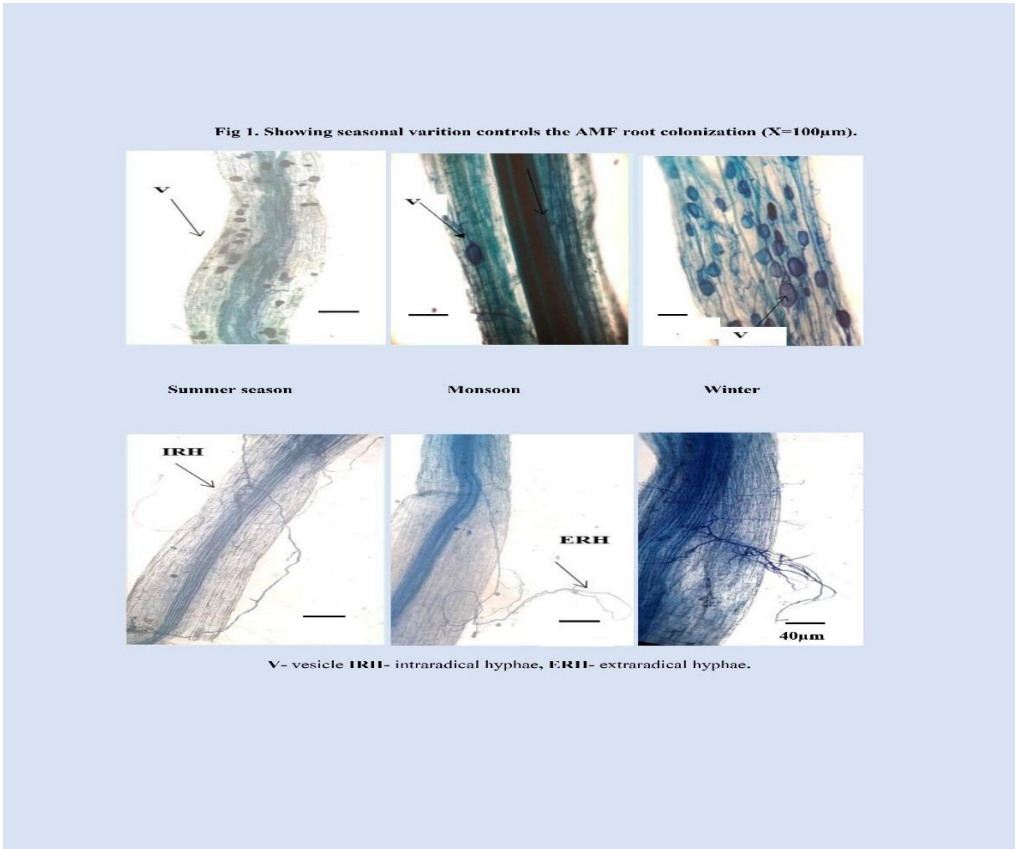
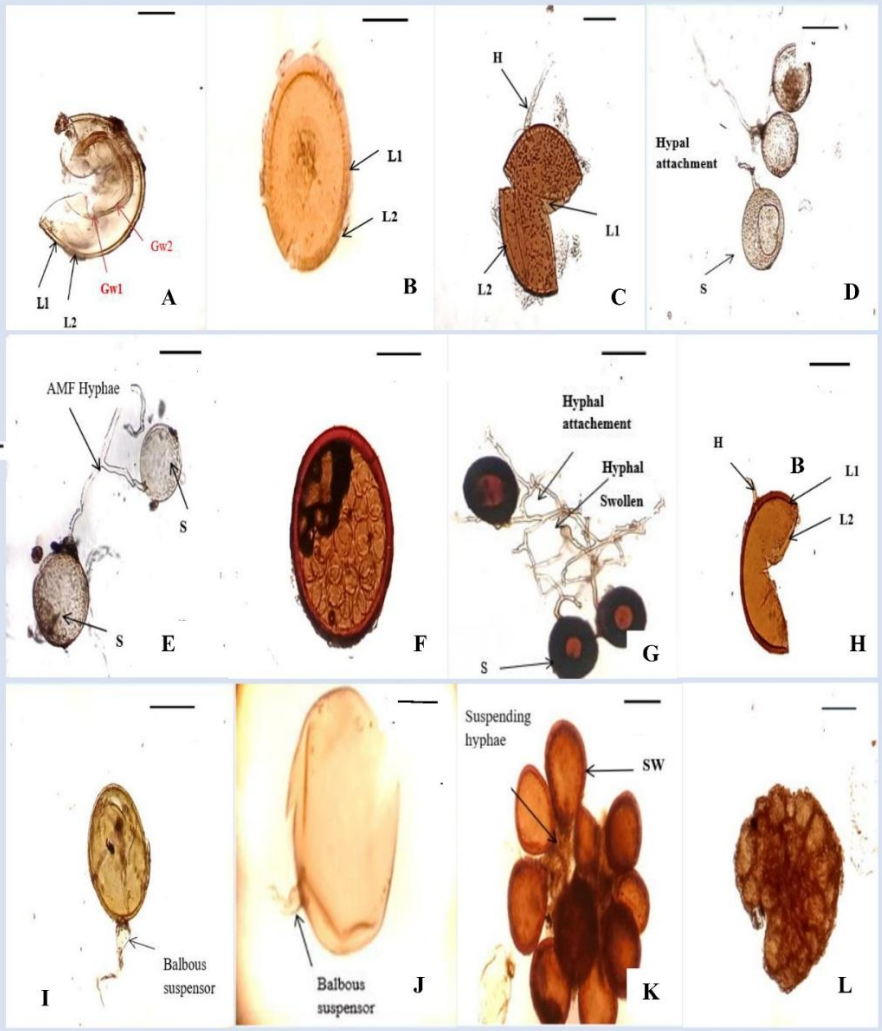


Fig 1. Showing seasonal varition controls the AMF root colonization (X=100µm).

V- vesicle IRH- intraradical hyphae, ERH- extraradical hyphae.

Fig. 2. Diversity of AM fungal spore isolated from the rhizosphere of *C. argentea.* (X=400)



A= *Acaulospora dilatate*, ***B***= *Acaulospora gerdemanii*, C= *Glomus citricola*, D=*Glomus clarum*, ***E***= *Glomus fasciculatum*, ***F***= *Glomus geosporum*, ***G***= *Glomus macroaggatum*, ***H***= *Glomus mosseae*, ***I***=*Gigaspora gigantea*, **J**= *Gigaspora margarita*, ***K***= *Sclerocystis rubiformis*, ***L***= *Sclerocystis sinuosa*

S- spore, L**1**-Layer 1, **L2-** layer 2, **H-** Hyphal, **Gw1-** Germinal wall 1, **Gw2 –** Germinal wall 2.

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