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Elemental analysis of mycorrhizal and actinorhizal inoculated *Casuarina equisetifolia* under glasshouse condition

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ABSTRACT

Casuarina equisetifolia is a diverse range of environments from tropical to arid woodlands and coastal sand dunes, salinity affected soils. *C. equisetifolia* is the only tree which is having tripartite symbionts in their roots system like endo, ectomycorrhizal and N_2 – fixing *Frankia*. Therefore the *C. equisetifolia* root system needs more amount of macro and micro elements for their metabolism. The present study aims to analyse the important 18 elements (N, P, K, Mg, Na, B, Al, Si, Cd, Ca, Fe, Co, Mo, Mn, Ni, Zn, Cr and Cu) which is essential for the *C. equisetifolia* phosphate solubilization and N_2 – Fixation. The results we have recorded that the cladodes (180 d) of triple (V + E + F) inoculated plants showed the higher amount of N, P, K, Mg and Na. Higher levels of N, K, Mg and Na were recorded in roots (180 d) of triple inoculated plants. Maximum P content was recorded in V + E inoculated plants. The stimulation of N_2 – fixation in the mycorrhizal plants can be attributed to increase in phosphate uptake by these roots, a situation analogous to that in nodulated legumes colonized by mycorrhizal fungi. Higher levels Ca, Fe, Co, Mn, Cu, Zn were recorded in cladodes (180 d) of triple (V + E + F) inoculated plants. Higher amount of Ni was recorded in cladodes of *Psilothus tinctorius* inoculated plants. Concentrations of Fe were higher in both foliage and twigs of *Casuarina equisetifolia*. These results prove that the triple inoculated (V + E + F) plants showed the enhanced elements in their cladodes and root system. Consequently this will boost the products of photosynthesis, and bio-mass of *Casuarina equisetifolia*.

KEYWORDS

Casuarina equisetifolia;
Glasshouse condition;
Endo and ectomycorrhiza;
Frankia;
Microelements;
Macroelements.

INTRODUCTION

Casuarina grows in a diverse range of environments, from tropical forest to arid woodlands and coastal dunes. Mycorrihizae were hypothesized to play an important role in plant growth in several dune restoration studies. *Casuarina* are capable of forming symbiotic nitrogen fixing associations with *Frankia* and may contribute significantly to the nitrogen economy of ecosystem. On the basis of morphology and cultural characteristics, the actinomycete endophytes of non-legumes are placed in one family *Frankiaceae* with a single genus, *Frankia*.

Physiological adaptations such as selective uptake and exclusion of elements by roots can influence the accumulation of minerals in tissues. Garten[2] maintained that tissue nutrient levels which reflect differential uptake, translocation, and storage reveal more about a species mineral element niche than soil nutrient levels. Mackkum[9] found the elemental differences between *Myrica* and *Chamaedaphne*. Small[12] analyzed leaf blades of both species from two bogs in Ontario for A1, Mn, N and P Langille and Maclean[8] analyzed leaves and twigs from two regions of Nova Scotia for Ca, Co, Cu, Mn, Mo, P and Zn. Macronutrients such as Mg and K, micronutrients such as B, and Fe are non essential but beneficial elements such as Na, Ni and Se serve important functions in plants[10] and are of interest when considering the overall nutrient status of different species. Information is also lacking on the allocation of elements between foliage and twigs in *Myrica* and *Chamaedaphne*. The quantity of foliar N resorbed in the fall can depend on site fertility and plant species, sites low in available N tend to have proportionately more N resorption from tree foliage in the fall transites high in available N[13]. Mycorrhizal infection may increase the uptake of copper[4] zink[7], nickel[6], chloride and sulphate[1].

Hence the present study aims to a Elemental analysis in effect of mycorrhizal and actinorhizal inoculation on *C. equisetifolia* under glasshouse condition.

MATERIALS AND METHODS

Shoots, cladodes and roots of *C. equisetifolia* were removed from the experimental from plants. Samples were taken to the laboratory on dry ice in insulated containers, dried in an oven at 65^0C.

Mineral analyses were made following the standard sample preparation and protocol for inductively coupled plasma spectrometry (McNaughton, 1990). One gram of each sample was dried and ashed in acid – washed crucilbles at 500^0C for 12h in 10ml of 2M HCl (spiked with at100 mg-g^{-1} for an internal standard) then vacuum – filtered using Millipore filter. Analyses of (18 elements) N, P, K, Mg, Na, B, Al, Si, Cd, Ca, Fe, Co, Mo, Mn, Ni, Zn, Cr and Cu were conducted 3410 Integrated Coupled Plasma Spectrophotometer with mini torch, Applied Research Lab (ARL) Switzerland.

RESULTS

Elemental analysis

The results of analyses of macroelements (N, P, K, Mg, Na, B, Al, Si and Cd) and Microelements (Ca, Fe, Co, Mo, Mn, Ni, Zn, Cr and Cu) of cladodes and roots are presented in TABLE 1-8.

Macroelements content of cladodes at 90 d

The results of macroelement content of cladodes at 90 d are presented in TABLE 1. The triple inoculated VAM + Ectomycorrhiza + *Frankia* (V+E+F) plants showed the maximum N-content (390.93 µg / g dry wt; +33%). The least amount of N-content was observed in *G. fasciculatum* treated plants (298.47; +1%). *Frankia* treated plants showed 335.87 µg (+14%) of N. The maximum amount of P was recorded in triple (V+E+F) inoculated plants (82.47;

TABLE 1 : Changes in macroelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 90 d.

Treatment	Microelements µg / g dry weight								
	N	P	K	Mg	Na	B	Al	Si	Cd
Conrol	294.31 ± 5.84	34.1 ± 5.46	198.03 ± 7.27	164.10 ± 3.78	94.57 ± 1.55	1.87±0.06	8.20 ± 0.17	19.30 ± 1.00	0.23 ± 0.06
G. fasciculatum (V)	298.47± 6.61	77.87±7.91	298.20±4.86	181.80±2.78	167.13±1.80	2.80±0.10	9.37±0.12	20.97±0.153	0.37±0.46
P. tinctorius (E)	311.77±9.10	75.01±6.29	296.77±5.02	214.33±3.41	175.60±65.09	2.53±0.06	9.40±0.36	21.17±0.81	0.16±0.02
Frankia (F)	335.87±0.16	65.97±1.81	310.33±3.95	262.43±1.50	279.23±18.40	3.00±0.10	8.73±0.29	16.20±0.17	0.60±0.20
V + E	331.47±25.72	67.93±4.10	334.60±10.34	234.77±5.16	279.27±3.86	3.10±0.10	9.33±0.06	19.33±1.05	0.19±0.01
V + F	352.53±12.99	65.43±13.81	333.47±29.03	243.17±2.61	282.53±8.46	2.97±0.06	9.37±0.12	21.10±0.35	0.31±0.01
E + F	373.27±11.83	80.20±7.17	503.47±8.78	264.40±21.15	272.47±9.96	3.57±0.21	5.27±0.06	25.20±0.17	0.28±0.01
V + E + F	390.93±13.31	82.47±10.93	510.13±12.25	282.10±16.32	289.07±7.09	3.77±0.23	6.63±0.29	24.97±1.15	0.10±0.02

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2^3- Factorial experiment showing the changes in macroelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 90 d.

Treatment	N	P	K	Mg	Na	B	Al	Si	Cd
Main effects									
G. fasciculatum (F)	0.142	44.743*	93.055*	4.804**	128.155*	76.488*	45.370*	5.048*	0.878
P. tinctorius (E)	2.554	39.265*	90.411*	38.697*	102.774*	39.024*	48.000*	6.332**	0.276
Frankia (F)	14.506*	23.720*	116.964*	148.285*	665.559*	112.780*	9.481*	17.464*	6.332**
2 Way Interaction									
V + E	11.611*	26.738*	172.975*	76.582*	665.800*	133.561*	42.815*	0.002	0.102
V + F	28.542*	22.933*	170.116*	95.870*	689.559*	106.244*	45.370*	5.888**	0.301
E + F	52.516*	49.641*	865.221*	154.275*	617.677*	253.756*	286.815*	63.259*	0.088
3 Way Interaction									
V + E + F	78.667*	54.643*	903.403*	213.530*	738.327*	316.976*	81.815*	58.354*	0.276

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

+141%) and least was recorded in V+F (65.43; +92%) inoculated plants. *G. fasciculatum* inoculated plants showed 77.87 µg (+128%) of P. Higher amount of K (510.13; +157%) was gtrecorded in triple (V+E+F) inoculated plants and least amount was recorded in *P. tinctorius* treated plants (2.53; +39). Higher amount (9.40; +15%) of Al was recorded in E+F reated plants. Higher level of Si (25.20; +30%) was recorded in E+F inoculated plants and least in *Frankia* treated plants (16.20; -16%). Maximum amount of Cd was estimated in *Frankia* treated plants (0.60; +160%) and least in V+E+F inoculated plants (0.10; -56%).

Macroelements of cladodes at 180 d

Higher amount of N was recorded in V+E+F inoculated plants (821.63; +44%) and least in *G. fasciculatum* inoculated plants (609.97; +7%) (TABLE 2). Higher amount of P was recorded in V+E+F inoculated plants (124.0; +103%) and least amount of P was in *Frankia* inoculated plants (98.30; +40%). Higher amount of K was recorded In V+E+F inoculated plants (719; +131%) and lower amount of K was in *G. fasciculatum* inoculated plants (418.70; +34%). More amount of Mg (410.53; +91%) and Na (364.50; +213%) was recorded in triple (V+E+F) inoculated plants and least amount of Mg (303.63; +41%) and Na (177.87; +52%) was in *G. fasciculatum* inoculated plants. Higher level of B was observed in *Frankia* inoculated plants (4.70; +88%) and least amount was in *G. fasciculatumi* inoculated plants (3.93; +57%). Higher amount of A1 (3.9; +54%) and Si (33.43; +19%) was recorded in triple inoculated plants. Least amount of A1 and Si was recorded in *P. tinctorius* (2.27; -12%) and *Frankia* inoculated plants (21.73; -22%), respectively. Higher amount of Cd was estimated in *G. fasciculatum* inoculated plants (0.69;+245%) and least was recorded in E+F inoculated plants, it was only 0.29; +45%).

TABLE 2 : Changes in macroelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d.

Treatment	Microelements µg / g dry weight								
	N	P	K	Mg	Na	B	Al	Si	Cd
Conrol	571.30±10.54	61.97±2.89	311.63±6.11	215.47±2.47	116.53±6.52	2.50±0.20	2.53±0.15	28.10±1.15	0.20±0.02
G. fasciculatum (V)	609.97±3.51	122.20±6.98	418.70±4.33	303.63±8.08	177.87±6.69	3.93±0.25	2.53±0.15	29.30±0.100	0.69±0.04
P. tinctorius (E)	621.87±09.45	114.13±5.80	511.87±4.83	315.13±3.53	190.33±5.95	4.63±0.31	2.27±0.15	28.40±0.85	0.29±0.03
Frankia (F)	802.17±10.09	98.30±2.00	619.13±4.86	329.30±2.65	200.00±3.16	4.70±0.46	2.47±0.64	21.73±1.40	0.34±0.08
V + E	694.17±5.31	118.47±1.76	635.80±5.07	371.47±10.20	290.23±3.10	4.60±0.26	3.13±0.40	25.47±4.65	0.37±0.05
V + F	709.90±3.62	110.67±9.22	688.67±4.38	383.33±11.00	306.13±4.54	4.50±0.20	3.77±0.49	30.00±2.04	0.38±0.05
E + F	719.13±4.22	108.37±1.81	711.30±4.58	338.67±46.56	336.07±6.52	4.53±0.47	3.70±0.56	31.37±1.90	0.29±0.07
V + E + F	821.63±9.61	124.20±9.99	719.97±3.51	410.53±5.92	364.50±5.11	4.67±0.32	3.90±0.10	33.43±3.46	0.33±0.11

2^3- Factorial experiment showing the changes in macroelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d

Treatment	N	P	K	Mg	Na	B	Al	Si	Cd
Main effects									
G. fasciculatum (F)	38.506*	161.598*	758.669*	37.035*	195.046*	29.233*	0	0.373	92.643
P. tinctorius (E)	65.854*	114.280*	265.483*	47.326*	282.394*	64.759*	0.705	0.023	3.361
Frankia (F)	137.694*	55.436*	625.974*	61.736*	361.218*	68.870*	0.044	10.498*	7.97**
2 Way Interaction									
V + E	388.794*	134.055*	695.729*	115.945*	156.381*	62.751*	3.570*	1.796	11.593*
V + F	494.741*	99.596*	940.124*	134.255*	186.887*	56.917*	15.085*	0.935	12.502*
E + F	562.854*	90.411*	57.567*	72.314*	249.871*	58.830*	13.499*	2.764	3.125
3 Way Interaction									
V + E + F	161.945*	152.356*	103.022*	181.288*	318.082*	66.798*	18.523*	7.367*	6.521**

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

Macroelements content of roots at 90 d

The macroelement contents of roots of experimental plants at 90 d are presented in TABLE 3. Higher amount of N (282.20 µg / g dry weight; +75%), P (42.60; +139%) and K (393.87; +95%) was recorded in triple inoculated (V+E+F) plants. The least amount of N (182.83; +13%), P (18.3; +3%) and K (203.63; +1%) was in VAM inoculated plans. Higher amount of Mg was recorded in V+F+F inoculated plants (151.90; +38%) and least amount was in *G. fasciculatum* inoculated (126.57; +15%) plants. Maximum amount of Na (97.30; + 134%) and B (3.85; +294%) was recorded in V+E+F inoculated plants. The least amount of Na (71.30; +73%)

TABLE 3 : Changes in macroelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 90 d.

Treatment	Microelements µg / g dry weight								
	N	P	K	Mg	Na	B	Al	Si	Cd
Conrol	161.40±8.97	17.87±1.69	201.60±9.07	109.57±3.16	41.53±1.37	0.97±0.01	1.70±0.26	9.50±1.64	0.24±0.06
G. fasciculatum (V)	182.83±8.20	18.30±1.73	203.63±3.79	126.57±4.74	71.30±1.00	1.21±0.11	1.33±0.25	10.63±1.53	0.14±0.07
P. tinctorius (E)	186.87±6.36	21.30±1.00	310.30±4.00	136.63±4.04	73.53±3.03	2.79±0.14	1.30±0.52	12.85±1.63	0.17±0.06
Frankia (F)	195.43±6.34	29.63±1.53	304.30±7.21	139.13±3.44	78.38±1.95	2.28±0.08	1.30±0.20	11.32±1.02	0.23±0.07
V + E	203.90±7.74	33.77±1.57	288.63±5.88	127.20±11.10	80.07±2.05	1.39±0.12	1.30±0.30	14.00±2.00	0.18±0.04
V + F	249.87±5.86	31.20±0.85	320.53±2.54	143.30±6.08	85.77±0.68	3.58±0.60	1.37±0.29	12.57±2.58	0.07±0.02
E + F	273.90±12.98	39.80±1.23	272.53±2.93	149.47±5.54	95.37±1.10	1.99±0.05	1.20±0.17	13.83±1.18	0.41±0.26
V + E + F	282.20±10.18	42.60±2.56	393.87±2.87	151.90±11.50	97.30±1.04	3.83±0.08	1.43±0.23	16.10±1.93	0.30±0.02

2^3- Factorial experiment showing the changes in macroelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 90 d.

Treatment	N	P	K	Mg	Na	B	Al	Si	Cd
Main effects									
G. fasciculatum (F)	9.268*	0.110	0.223	9.036*	466.549*	1.651	2.180	0.636	1.440
P. tinctorius (E)	13.084*	6.910*	637.375*	22.905*	539.184*	94.247*	2.595	5.570**	0.661
Frankia (F)	23.367*	81.165*	568.953*	27.331*	713.071*	49.188*	2.595	1.641	0.037
2 Way Interaction									
V + E	36.439*	148.203*	408.608*	9.721*	781.826*	233.901*	2.598	10.051*	0.599
V + F	157.889*	104.218*	763.032*	35.578*	103.235*	194.753*	1.802	4.658**	3.897
E + F	255.328*	282.015*	271.417*	49.774*	152.947*	29.820*	4.054	9.301*	3.745
3 Way Interaction									
V + E + F	294.393*	358.615*	199.086*	56.030*	163.519*	4.976**	1.153*	21.577*	0.384

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

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and B (1.21; +33%) was in *G. fasciculatum* inoculated plants. Compared to other treatments least amount of Al (1.20; -29%) was recorded in E+F plants. Least amount of Si (10.63; +11.0%) was in *G. fasciculatum* inoculated plants. V+F inoculated plants accumulated least amount of Cd (0.07; -10%).

Macroelements of roots at 180 d

Changes in contents of macroelements of roots of experimental plants at 180 d were presented in TABLE 4. Higher amount of N (650.50 µg; +63%), K (479; +180%), Mg (190; +54%), Na (127.77; +108%) was in triple (V+E+F) inoculated plants and maximum P content (51.50; +59%) was in V+E plants. Least amount of N (418.23; +5%), P (38.13; +19%), K (392.23; +88%), Na (81.97; +32%) and Mg (139.77; +13%) was in *G. fasciculatum* inoculated plants. Higher level of B (4.34; +104%) was recorded in *Frankia* inoculated plants. Least amount of B (2.90; +38%) was in V+F inoculated plants. Maximum level of Al (4.46; +64%) and Si (15.90; +53%) was recorded in V+E inoculated plants. Least amount of Al (3.20; +45%), Si (13.24; +27%) was recorded in *Frankia* and E+F inoculated, respectively. No Cd was recorded in *P. tinctorius*, *Frankia*, V+F, E+F, V+E+F inoculated plants. In *G. fasciculatum* inoculated plants, least amount of Cd (0.25; -24%) was recorded and (0.80; +142%) was in V+E inoculated plants.

TABLE 4 : Changes in macroelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d.

Treatment	Microelements µg / g dry weight								
	N	P	K	Mg	Na	B	Al	Si	Cd
Conrol	398.97±3.21	32.23±1.07	208.23±2.10	123.83±3.95	61.30±1.00	2.15±0.05	2.20±0.05	10.47±0.04	0.33±0.02
G. fasciculatum (V)	418.23±4.29	38.13±0.86	392.23±8.85	139.77±1.59	81.97±2.08	3.27±0.06	4.46±0.56	14.46±10.56	0.25±0.04
P. tinctorius (E)	437.97±4.93	47.47±0.76	402.53±2.58	150.13±1.95	94.73±4.18	3.72±0.02	3.60±0.02	13.41±0.10	0
Frankia (F)	491.47±4.86	47.20±1.01	394.07±2.75	165.57±6.84	86.97±2.47	4.34±0.06	3.20±0.02	14.44±0.23	0
V + E	551.43±3.11	51.50±1.53	398.07±2.75	168.87±3.39	110.97±2.52	4.01±0.01	3.71±0.02	5.90±1.24	0.80±0.02
V + F	591.20±4.71	49.63±1.06	434.43±5.22	166.93±3.56	106.30±1.00	2.90±0.05	3.53±0.05	15.85±0.46	0
E + F	630.03±1.91	44.83±0.81	466.27±3.65	187.93±4.01	120.30±2.00	3.75±0.05	3.44±0.03	13.24±0.12	0
V + E + F	650.50±2.00	48.40±1.23	479.57±2.97	190.30±2.00	127.77±0.92	3.76±0.05	3.25±0.05	13.56±0.07	0

2^3- Factorial experiment showing the changes in macroelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d.

Treatment	N	P	K	Mg	Na	B	Al	Si	Cd
Main effects									
G. fasciculatum (F)	38.331*	45.819*	452.539*	26.949*	124.775*	836.267*	195.444*	89.225*	40.421*
P. tinctorius (E)	157.060*	305.446*	504.499*	73.426*	326.547*	165.252*	274.525*	48.431*	673.965*
Frankia (F)	883.530*	294.846*	461.879*	184.885*	192.453*	320.141*	140.778*	88.213*	673.965*
2 Way Interaction									
V + E	240.422*	398.514*	481.098*	215.280*	720.636*	230.400*	319.914*	165.175*	5.684*
V + F	381.884*	488.605*	684.941*	191.741*	591.577*	378.341*	2477.837*	162.143*	673.965*
E + F	551.309*	208.971*	890.891*	436.167*	101.928*	171.785*	217.058*	42.986*	673.965*
3 Way Interaction									
V + E + F	653.243*	344.022*	984.215*	468.969*	120.607*	173.230*	156.031*	53.504*	673.965*

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

Microelements of cladodes at 90 d

Level of microelements of Cladodes at 90 d is presented in TABLE 5. Higher level of Ca (11.70 µg / g dry wt; +85%), Fe (32.70; +140%), Co (0.39; +15%), Mo (1.63; +101%), Mn (4.70; +292%), Zn (0.48; +118%) and Cu (0.39; +50%) was recorded in triple (V+E+F) inoculated plants. The least amount of Ca (6.93; +9%), and Mn (2.63; + 119%) was in *G. fasciculatum* inoculated plants. Minimum level of Mo (0.86; +6%), Cu (0.28; +8%) was in *P. tinctorius* inoculated plants.

The maximum amount of Ni (0.26; +30%) was recorded in *P. tinctorius* inoculated plants, and least (0.16;

TABLE 5 : Changes in microelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 90 d.

Treatment	Microelements µg / g dry weight								
	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Conrol	6.37±0.91	13.60±2.86	0.34±0.40	0.81±0.04	1.20±0.10	0.20±0.02	0.22±0.03	0.72±0.03	0.26±0.03
G. fasciculatum (V)	6.93±0.60	16.60±0.70	0.20±0.01	0.96±0.02	2.63±0.21	0.24±0.01	0.19±0.02	0.34±0.03	0.30±0.03
P. tinctorius (E)	8.87±1.25	19.20±1.05	0.20±0.02	0.86±0.04	2.73±0.21	0.26±0.04	0.51±0.03	0.36±0.12	0.28±0.02
Frankia (F)	9.33±0.79	18.90±2.46	0.32±0.02	1.33±0.25	3.10±0.30	0.23±0.02	0.35±0.17	0.62±0.03	0.31±0.01
V + E	8.40±0.92	17.93±1.52	0.33±0.03	0.98±0.07	3.27±0.21	0.25±0.01	0.14±0.02	0.35±0.02	0.29±0.02
V + F	8.10±0.20	19.97±1.53	0.37±0.03	1.11±0.09	0.87±0.15	0.18±0.02	0.27±0.06	0.23±0.02	0.38±0.05
E + F	10.73±0.47	27.73±1.07	0.33±0.06	1.18±0.11	4.50±0.26	0.16±0.02	0.46±0.04	0.27±0.05	0.36±0.02
V + E + F	11.70±0.20	32.70±0.78	0.39±0.02	1.63±0.04	4.70±0.26	0.22±0.02	0.48±0.03	0.37±0.03	0.39±0.02

2^3- Factorial experiment showing the changes in microelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 90 d.

Treatment	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Main effects									
G. fasciculatum (F)	0.857*	4.858*	1.335	2.935	62.678*	5.319**	0.377	83.458*	3.740
P. tinctorius (E)	16.679*	16.928*	1.335	0.419	71.729*	14.729*	28.242*	77.654*	1.273
Frankia (F)	23.487*	15.163*	0.020	89.136*	110.136*	3.560	5.439**	5.497**	6.649
2 Way Interaction									
V + E	11.033*	10.136*	0.007	3.919	130.305*	14.242*	2.354	80.529*	2.104
V + F	8.018**	21.881*	0.096	11.739*	216.949*	2.813*	0.964	139.320**	33.662*
E + F	15.885*	107.828*	0.001	18.180*	332.237*	6.330**	18.987*	12.889*	24.961*
3 Way Interaction									
V + E + F	75.908*	196.928*	0.203*	35.270*	373.729*	0.703	23.507*	73.438*	37.506*

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

-20%) was in E+F inoculated plants. Maximum level of Zn (0.51; +131%) was observed in V+E inoculated plants. Least amount of Cr (0.23; -68%) was in V+F inoculated plants.

Microelements of cladodes at 180 d

Changes in microelements content of cladodes at 180 d are presented in TABLE 6. Higher level of Ca (23.43 µg/g dry wt; +146), Fe (32.54; +113%), Co (0.58; +190%), Mn (6.53; +170%), Cu (0.46; +100%) and Zn (0.86; +166%) were recorded in triple (V+E+F) inoculated plants. Least amount of Ca (10.3; +50%), Fe (23.13; +52%), Co (0.22; +10%), Mn (3.53; +45%) and Cu (0.33; +50%) were recorded in *G. fasciculatum* inoculated plants. Maximum level of Mo (2.68; +116%) was recorded in *Frankia* inoculated plants and least (0.22; -83%) in *G. fasciculatum* inoculated plants. Higher amount of Ni (0.84; +162%) was recorded in *P.*

TABLE 6 : Changes in microelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d.

Treatment	Microelements µg / g dry weight								
	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Conrol	9.53±0.68	15.27±0.68	0.20±0.02	1.27±0.25	2.40±0.01	0.32±0.01	0.39±0.02	0.23±0.02	0.29±0.02
G. fasciculatum (V)	10.37±0.40	23.13±1.39	0.22±0.02	0.21±0.01	3.53±0.31	0.22±0.03	0.64±0.05	0.22±0.02	0.33±0.03
P. tinctorius (E)	11.60±0.61	24.63±1.76	0.30±0.02	1.30±0.02	4.40±0.17	0.84±0.03	0	0.24±0.02	0.39±0.03
Frankia (F)	12.17±1.62	27.24±3.13	0.30±0.02	2.68±0.14	4.50±0.20	0.53±0.03	0.45±0.02	0.23±0.02	0.34±0.03
V + E	12.17±1.62	24.87±0.51	0.50±0.02	1.81±0.02	4.17±0.15	0.42±0.02	0.30±0.02	0	0.34±0.03
V + F	18.30±2.00	30.13±1.61	0.54±0.02	1.82±0.06	5.30±0.26	0	0	0	0.40±0.02
E + F	20.30±5.29	31.90±2.14	0.52±0.05	1.83±0.06	5.43±0.15	0.29±0.02	0	0.13±0.02	0.42±0.01
V + E + F	23.43±4.87	32.54±1.35	0.58±0.02	0.90±0.02	6.53±0.25	0	0.86±0.02	0.10±0.02	0.46±0.02

2^3- Factorial experiment showing the changes in microelements content of cladodes of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d

Treatment	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Main effects									
G. fasciculatum (F)	0.140	30.012*	1.298	148.269*	43.623*	137.378*	185.950*	0.571	3.533
P. tinctorius (E)	0.861	42.562*	27.126*	0.179	98.151*	108.600*	460.298*	0.254	28.058*
Frankia (F)	0.495	69.547*	27.126*	266.507*	149.774*	170.844	8.463*	0.063	5.723*
2 Way Interaction									
V + E	1.398	44.709*	224.212*	38.722*	106.000*	40.000*	24.099*	302.286*	5.723*
V + F	15.499*	107.220*	51.285*	40.658*	285.623*	409.600*	460.298*	302.286*	29.898*
E + F	23.378*	134.217*	936.265*	41.644*	312.491*	2.844	460.298*	57.143*	44.409*
3 Way Interaction									
V + E + F	38.965*	144.80*	112.132*	17.853*	580.226*	409.600*	647.934*	101.587*	75.942*

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

tinctorius inoculated plants and Ni was not located in V+E+F and V+F inoculated plants. Zn was not recorded in *P. tinctorius,* V+E, V+F and E+F inoculated plants. Cr was not observed in V+E and V+F inoculated plants.

Microelements of roots at 90 d

Changes in microelements content in the roots of inoculated plants at 90 d are presented in TABLE 7. Higher level of Ca (3.77 µg/g dry weight; +208%), Co (0.75; +300%), Mo (2.70; +250%) and Fe (5.57; +56%) were registered in triple (V+E+F) inoculated plants. Least amounts of Ca (2.27; +83%), Fe (5.07; +5%), Co (0.24; +84%) and Mo (0.38; +50%) were recorded in *G. fasciculatum* inoculated plants. Maxi-

TABLE 7 : Changes in microelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 90 d.

Treatment	Microelements µg / g dry weight								
	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Conrol	1.27±0.25	4.83±0.50	0.13±0.02	0.24±0.06	1.27±0.31	0.14±0.04	0.33±0.03	0.37±0.04	0.30±0.02
G. fasciculatum (V)	2.27±0.46	2.07±0.07	0.24±0.07	0.38±0.06	1.40±0.10	0.34±0.05	0.33±0.03	0.59±0.06	0.30±0.02
P. tinctorius (E)	2.97±0.21	6.20±0.85	0.27±0.05	0.41±0.05	2.03±0.25	0.35±0.07	0.46±0.06	0.43±0.01	0.43±0.02
Frankia (F)	2.80±0.72	6.60±0.66	0.14±0.07	0.39±0.02	2.73±0.42	0.31±0.10	0.43±0.01	0.40±0.02	0.42±0.03
V + E	2.37±0.72	6.60±0.66	0.35±0.06	0.50±0.02	2.87±0.67	0.35±0.04	0.29±0.06	0.57±0.06	0.38±0.10
V + F	3.20±0.35	5.67±0.55	0.72±0.10	0.63±0.08	2.80±0.61	0.40±0.07	0.25±0.04	0.34±0.11	0.34±0.04
E + F	3.58±0.38	6.60±1.65	0.68±0.06	0.68±0.06	3.03±0.15	0.32±0.05	0.34±0.07	0.22±0.02	0.39±0.01
V + E + F	3.77±0.51	7.57±0.67	0.75±0.02	2.70±0.61	0.27±0.06	0.31±0.01	0.31±0.01	0.37±0.06	0.39±0.03

2^3- Factorial experiment showing the changes in microelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d.

Treatment	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Main effects									
G. fasciculatum (F)	8.511*	0.118	4.571**	11.225*	0.138	17.593*	0	2.954	0.010
P. tinctorius (E)	24.596*	4.043	8.254**	18.057*	4.560**	19.366*	13.641*	25.851*	14.192*
Frankia (F)	20.009*	9.246	0.018	11.780*	16.690*	16.690*	9.184*	933.646*	12.039*
2 Way Interaction									
V + E	10.298*	6.756**	19.446*	40.628*	19.862*	18.766*	0.897	4.615**	5.199
V + F	31.811*	1.503	138.286*	92.982*	18.241*	28.033*	4.341	50.262*	1.415**
E + F	43.726*	6.756**	121.540*	114.598*	24.216*	14.303*	0.081	92.082*	7.165*
3 Way Interaction									
V + E + F	53.191*	16.173*	152.790*	146.270*	15.940*	6.827**	0.143	39.713*	7.705**

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

mum amount of Mn (3.03; +150%) was recorded in E+F inoculated plants minimum of Mn (1.40; +10%) was recorded in *G. fasciculatum* inoculated plants. Higher level of Ni (0.40; +300%) was recorded in V+F inoculated plants and least (0.27; +100%) was in triple inoculated (V+E+F) plants. Higher level of Zn (0.46; +33%) was recorded in *P. tinctorius* inoculated plants. Higher amount of Cr (0.59; +59%) was recorded in *G. fasciculatum* inoculated plants and least (0.22; -40%) was in the E+F inoculated plants. Maximum amount of Cu was recorded in *P. tinctorius* inoculated plants (0.43; +33%) and least was in *G. fasciculatum* (0.34; +10%) inoculated plants.

Microelements of roots at 180 d

Changes in microelements of roots of experimental plants at 180 d are presented in TABLE 8. Higher levels of Ca (4.30 µg / g dry weight ; +104%), Fe (12.30; +92%) and Co (0.29; +100%) were recorded in triple inoculated (V+E+F) plants. Least amount of Ca (2.61; +24%) and Fe (4.30; -32.6%) was recorded in *G. fasciculatum* and *P. tinctorius* inoculated plants, respectively. Least level of Co (0.12; -25%) and Higher amount of Mo (1.66; +220%) were recorded in *Frankia* inoculated plants. Least amount of Mo (0.41; +33%) was observed in *G. fasciculatum* inoculated plants. Higher level f Mn (4.30; +104%) and Ni (0.45; +100%)

TABLE 8 : Changes in microelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d.

Treatment	Microelements µg / g dry weight								
	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Conrol	2.14±0.07	6.48±0.03	0.16±0.02	0.30±0.02	2.16±0.02	0.22±0.22	0.18±0.03	0.11±0.01	0.18±0.02
G. fasciculatum (V)	2.61±0.04	7.91±0.02	0.23±0.03	0.41±0.09	3.14±0.04	0.30±0.02	0.40±0.03	0	0.16±0.02
P. tinctorius (E)	3.18±0.05	4.30±0.02	0.29±0.01	0.52±0.02	4.30±0.01	0.42±0.01	0.22±0.02	0.22±0.02	0.18±0.02
Frankia (F)	3.81±0.10	8.10±0.03	0.12±0.02	0.96±0.04	3.23±0.05	0.45±0.02	0.33±0.01	0.17±0.01	0.17±0.01
V + E	3.69±0.02	9.29±0.02	0.29±0.02	0.50±0.03	2.66±0.04	0.31±0.03	0.24±0.03	0	0.20±0.02
V + F	3.96±0.02	10.67±0.15	0.20±0.02	0.92±0.03	2.04±0.04	0.20±0.02	0.42±0.02	0.12±0.01	0.19±0.01
E + F	4.15±0.05	11.23±0.21	0.26±0.06	0.74±0.03	2.40±0.02	0.35±0.04	0.30±0.02	0.14±0.02	0.19±0.01
V + E + F	4.30±0.02	12.30±0.02	0.29±0.02	0.49±0.03	2.66±0.04	0.10±0.02	0.30±0.02	0	0.17±0.04

2^3- Factorial experiment showing the changes in microelements content of roots of mycorrhizal and actinorhizal inoculated *C. equisetifolia* at 180 d.

Treatment	Ca	Fe	Co	Mo	Mn	Ni	Zn	Cr	Cu
Main effects									
G. fasciculatum (F)	122.157*	350.927*	8.859*	10.496*	135.859*	17.323*	222.281*	121.000*	2.813
P. tinctorius (E)	598.120*	819.379*	33.595*	40.723*	646.318*	111.910*	611.438*	113.778*	0.044
Frankia (F)	154.249*	454.347*	3.534	160.472*	159.251*	147.368*	350.711*	40.111*	0.703
2 Way Interaction									
V + E	131.167*	135.169*	35.436*	35.865*	348.251*	23.579*	593.587*	121.000*	0.703
V + F	183.742*	302.100*	2.454	333.455*	20.329*	1.083	190.942*	1.778	0.176
E + F	222.753*	389.548*	22.086*	165.407*	76.863*	48.120*	414.678*	9.000	0.176
3 Way Interaction									
V + E + F	257.098*	583.381*	33.595*	122.552*	348.251*	34.767*	429.620*	121.000*	0.396

***Significance at $p < 0.001$; **Significance at $p < 0.05$**

Shoot

Source of Variation	DF	Mean Square	F-Value	Sinif. of F
Nutri	17	13330790.299	370.948	0
Treat	7	34847.886	9.714	0
Duration	1	387109.970	107.904	0.003
Residual	838	3587.536		
Total	863	30429.705		

Root

Source of Variation	DF	Mean Square	F-Value	Sinif. of F
Nutri	17	640906.469	345.700	0
Treat	7	10826.177	5.840	0
Duration	1	156518.803	84.425	0.003
Residual	838	1853.938		
Total	863	14694.452		

were recorded in *P. tinctorius* and *Frankia* inoculated plants, respectively. Higher amount of Zn (42.00; +100%) was recorded in *G. fasciculatum* inoculated plants. Higher (0.22; +100%) and lower level (0.12; +10%) of Cr were recorded in *P. tinctorius* and V+F inoculated plants. Higher amount of Cu (0.16; -50%) was recorded in *G. fasciculatum* inoculated plants.

DISCUSSION

The cladodes (180 d) of triple inoculated (V+E+F) plants showed higher amount of N, P, K, Mg and Na. Higher levels of N, K, Mg and Na were recorded in roots (180 d) of triple (V+E+F) inoculated plants. Maximum P content was recorded in V+E inoculated plants. The stimulation of N_2 fixation in the mycorrhizal plants can be attributed to increase in phosphate uptake by these roots, a situation analogous to that in nodulated legumes colonized by mycorrhizal fungi. Distribution of biomass and N among plant parts and soil N in a young *Alnus incana* stand has been demonstrated[5].

Higher levels of Ca, Fe, Co, Mn, Cu, Zn were recorded in cladodes (180 d) of triple (V+E+F) inoculated plants. Higher amount of Ni was recorded in cladodes of *P. tinctorius* inoculated plants. High levels of Ca, Fe and Co were recorded in roots (180 d) of triple inoculated plants. Mackkum[9] analysed the foliage of *C. calyculata* with reference to Al, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Se, V and Zn. *C. caliculata* foliage had maximum Al, Mn, P and Cu, whereas *M. gale* foliage had maximum K, Ni, Mo and Zn. Physiological adaptations such as selective uptake and exclusion of elements by roots can influence the accumulation of minerals in tissues. Garten[2] maintains that tissue nutrient levels, which reflect differential uptake, translocation and storage reveal more about a species mineral element niche than soil nutrient levels.

Concentrations of Fe were higher in both foliage and twigs of *M. gale* than in *C. calyculata* tissue. Fe is required for photosynthesis as a component of cytochromes, as well as in the biosynthesis of chlorophyll[10] and photosynthetic rates were shown to be higher in deciduous species than in evergreen sclerophylls[12]. K influences stomatal movement by causing turgor changes in guard cells[10]. Therefore, high foliar concentrations of K in *Myrica* compared with *Chamaedaphne* might reflect the different water relations of a deciduous and an evergreen sclerophyllous shrub. Higher level of K in foliage of triple inoculated plants revealed that *C. equisetifolia* has drought tolerance capacity.

Ni is essential in legumes that translocate large quantities of N from roots to shoots as ureides. The ureides break down to form urea, which must be hydrolysed by a urease that requires Ni, for the urea-N to be utilized. Interestingly both in cladodes and roots of *P. tinctorius* inoculated *C. equisetifolia*, higher level of Ni was observed. Langille and Maclean[8] found higher concentrations of Mo and Zn in current growth of *Myrica* than in *Chamaedaphne*. Mo is a component of nitrogenase and is required for N-fixation in legumes as well as non-legumes[10]. Frankia inoculated plants showed high level of Mo. Zn is a component of enzymes and ribosomes and has been associated with protein synthesis[10]. Cladodes of triple inoculated *C. equisetifolia* contained more amount of Zn.

ABBREVIATIONS

VAM - Vesicular Arbuscular Mycorrhiza
V - *Glomus fasciculatum*
E - *Psilothus tinctorius*
F - *Frankia*

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