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Research article

Arbuscular mycorrhizal fungi inoculation with organic matter and phosphorus supplementation enhance nutrient contents of *Amaranthus tricolor* L. and *Basella alba* L. by improving nutrients uptake

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Abstract: Most of the vegetable crops retain an association with arbuscular mycorrhizal fungi (AMF) which can improve the growth, nutrition, water supply and tolerance to biotic and abiotic stresses of host plants. Therefore, the main objective of this study was to investigate whether the association of red amaranth (*Amaranthus tricolor*) and Indian spinach (*Basella alba*) with AMF, increase the shoot mineral nutrient contents and whether supplementation of organic matter and phosphorus enhance AMF association. The experiment was laid out in a factorial design considering three-factor *viz.* arbuscular mycorrhiza (AM), cowdung (CD) and phosphorus (P). There were five replications and total 40 treatment combinations. Results showed that in both vegetable maximum N, P, K, S, Mg, Fe, Mn, Cu and Zn and protein contents were observed due to the application of AMF and cowdung with phosphorus. The combined application of AMF with cowdung and phosphorus enhanced the association with plants and was more effective than only AMF application to enhance nutrient contents. Also, the uptake of almost all macro- and micronutrients increased due to the application of cowdung and phosphorus with arbuscular mycorrhiza.

Keywords: Arbuscular mycorrhizal fungi - Mineral nutrients - Red amaranth - Indian spinach - Nutrient uptake - Cowdung - Phosphorus.

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INTRODUCTION

The utilization of natural resources for sustainability and exponential increase in agricultural production has now become a demand of the globalized era to fulfil the nutritional needs of the thriving population. The phenomenon of arbuscular mycorrhizal symbiosis has paved the way towards a new window of enhanced agricultural production as well as nutritional security. All arbuscular mycorrhizal fungi (AMF or AM fungi) are obligate symbionts and are grouped in the phylum Glomeromycota, with almost 220 species actually described with the majority of representatives belong to the order Glomerales (Schüßler *et al.* 2001).

The diversity of AM fungal community may have an influence on plant communities and various fungal species preferentially associate with different plant species (Pringle & Bever 2002). AMF form a symbiotic and mutualistic interconnection with thin roots of most terrestrial plants (Yoshimura *et al.* 2013). The AMF mycelium interacts with the plant roots and helps plants to uptake more nutrients from soil-water system that are distant from roots (Smith *et al.* 2000). Furthermore, fungal hyphae are much thinner than roots and are which facilitated to penetrate smaller pores (Allen 2011).

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The symbiotic relation provides plant many benefits, including enhanced uptake of poorly mobile soil nutrients and reduced susceptibility of roots to soil-borne pathogens (Quilambo 2003). The fungus also receives carbohydrate and growth factors from the plant (Berruti *et al.* 2016). AMF hyphae exclusively colonize the root cortex and form highly branched structures inside the cells, *i.e.*, arbuscules, which are considered the functional site of nutrient exchange (Balestrini *et al.* 2015). Consequently, AMF association helps to update adequate nutrients and enhance plant growth (Nouri *et al.* 2014).

Mycorrhizal symbioses not only profit to plant growth but also to plant protection, especially against environmental stresses. Scarcity of essential nutrients especially phosphorus is primarily limits crop growth and productivity (Nagarathna *et al.* 2007). The AMF have been suggested as having a role in mediating the uptake of water at times of drought stress and of metals on the contaminated ground (Farahani *et al.* 2008). The scarcity of inorganic fertilizers in some poor developing countries considerably hamper crop cultivation. The incorporation of factors that enable plants to withstand nutrient deficiency and toxicity as well as drought stress would, therefore, be helpful to improve crop production.

Leafy vegetables that lack natural AMF association as such red amaranth and Indian spinach, can be incorporated with the inoculation of AMF along with cowdung and phosphorus to increase their nutrient content. AMF accept the hexoses sugars from the host plant. Carbon transfer occurs from plant to fungi may occur through the arbuscular or intraradical hyphae. The host plants invest considerable carbon in the mycorrhizal network and contribute to the below-ground organic carbon pool. The main benefit of mycorrhizas to plants has been attributed to increased uptake of nutrients, especially phosphorous.

The level of minerals in vegetables depends on a number of factors including genetic properties of the crop species, climatic conditions, soil characteristics and the degree of maturity of the plant at the moment of harvesting (Carvajal *et al.* 2014). As vegetables constitute the main source of minerals in the human diet, one of the most important challenges for agriculture, besides enhancing food production, is to provide almost all the essential minerals and organic nutrients to humans for maintenance of health and proper organ function. Humans need more than 22 mineral elements. Some are required in large amounts, such as Fe, Zn, Cu, I and Se, some are required in trace amounts (Welch & Graham 2004) but their absence renders human life impossible. Concentrations of several essential elements in modern crops are insufficient for optimal human nutrition, thus contributing to the huge “hidden hunger” problem (Graham *et al.* 2001). This is the case of iron (Fe), whose deficiency affects more than 3.5 billion people, mostly in the developing world and impairs the cognitive development of children, causes productivity and educational losses and increases morbidity and maternal mortality (ACC/SCN 2000). Other important examples are zinc (Zn), a micronutrient whose levels of intake in the diet may be inadequate for nearly half of the world’s population and copper (Cu), essential for human health but consumed in inadequate quantities in developing countries (Brown & Wuehler 1990).

Considering the above-mentioned facts, this study was carried out to evaluate the potential of arbuscular mycorrhizal fungi inoculation with organic matter and phosphorus supplementation to produce mineral nutrients rich leafy vegetables *i.e.* red amaranth and Indian spinach that lack natural arbuscular mycorrhizal fungi association.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the net house of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh.

Collection and preparation of soil sample

The soil used in this experiment was collected from Central Farm of Bangladesh Agricultural University, Mymensingh. The soil was dried and the clods were broken and sieved to remove weeds, stubbles and hard clods. Before starting the experiment, the soil was analyzed primarily for its physical and chemical properties. Organic carbon, soil pH, soil texture, cation exchange capacity, total nitrogen, available phosphorus, exchangeable potassium, available sulphur were analyzed (Table 1).

Table 1. Different properties of experimental soil.

pH	CEC (%)	Total N (%)	Organic carbon (%)	Organic matter (%)	Available P (μg g^{-1})	Exchangeable K (cmol kg^{-1})	Available S (μg g^{-1})
6.18	8.4	0.10	0.71	1.16	12.6	0.14	10.9

Analysis of cowdung

Cowdung manure was analyzed for assessing the constituents of N, P, K, S, Ca, Mg and Fe following the standard methods used for analysis. Total N (%)=1.28, P (%)=0.60, K (%)=1.65, Ca (%)=0.43, Mg (%)=0.28, S (%)=0.16, Fe(%)=14.47.

Test crops

Red amaranth cv. BARI Lalshak-1 and Indian spinach cv. BARI Puishak-1 were used as plant materials for the experiment. The seeds were collected from Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

Treatment combination and experimental design

There were eight treatment combinations for each test crop. The experiment was laid out in a factorial design considering three factor arbuscular mycorrhiza (AM), cowdung (CD) and phosphorus (P). There were five replications and total treatment combinations were 40. T_1=Control, T_2 = AM, T_3=CD, T_4=P, T_5=AM+CD, T_6=CD+P, T_7=AM+P, T_8=AM+CD+P. (Here, AM= Arbuscular mycorrhiza, CD= Cowdung, P= Phosphorus).

Fertilizer dose- For red amaranth: AM=80 g polybag^{-1}, CD= 20 g polybag^{-1}, P=0.30 g TSP polybag^{-1}

Basal dose- Urea @ 110 kg ha^{-1} (0.95 g polybag^{-1}), MOP @ 25 kg ha^{-1} (0.20 g polybag^{-1}) and Gypsum @ 4 kg ha^{-1} (89 mg polybag^{-1})

For Indian spinach: AM=80 g polybag^{-1}, CD= 32 g polybag^{-1}, P=0.30 g TSP polybag^{-1}

Basal dose- Urea @ 120 kg ha^{-1} (1.04 g polybag^{-1}), MOP @ 50 kg ha^{-1} (0.40 g polybag^{-1}) and Gypsum @ 13 kg ha^{-1} (290 mg polybag^{-1})

Per polybag 8 kg soil was used. Basal doses were added from Fertilizer Recommendation Guide-2005.

Application of arbuscular mycorrhizal (AM) fungi in soil and sowing of seed

Soil-based AM inoculum was used in the seed furrows of about 3 cm depth at the rate of 80 g polybag^{-1}. The seeds of each test crop were sown in each furrow on AM inoculums and covered them with side soil. After sowing the seed, the soil was saturated with water.

Preparation of the plant extract for different chemical analyses

Plant extract was prepared by wet oxidation method using a di-acid mixture (Estefan *et al.* 2013). This solution was used for the determination of P, K, S, Ca, Mg, Fe, Mn, Cu and Zn.

Analytical methods

The phosphorus content was determined by spectrophotometer (Model- LT-31) at 660 nm wavelength (Page *et al.* 1987). The content of K was determined separately with the help of flame emission spectrophotometer (Model- Jenway PET 7) (Ghosh *et al.* 1983). Calcium and magnesium content was determined by the complexometric method of titration using Na_2EDTA (Page *et al.* 1987). Sulphur content was analyzed turbidimetrically with the help of spectrophotometer (Model- LT-31) as described by (Wolf 1982) and (Tandon 1995). The contents of Fe, Zn, Cu and Mn were determined by atomic absorption spectrophotometer setting wavelengths at 248.3, 213.9, 324.8, 279.5 nm, respectively (Allen *et al.* 1974). Total N was estimated by semi-micro Kjeldahl method (Jackson 1973, Page *et al.* 1987).

i. *Calculation of protein content*

A protein present in plant samples was calculated by the following formula.

$$\text{Protein (\%)} = \text{\% Nitrogen} \times 5.5$$

ii. *Nutrient uptake*

After chemical analyses of plant samples, the nutrient contents were calculated and from the value of nutrient contents, nutrient uptakes were also calculated by the following formula:

$$\text{Nutrient uptake (mg plant}^{-1}) = \frac{\text{Nutrient content (\%)} \times \text{Yield (mg plant}^{-1})}{100}$$

Statistical analyses

Analysis of variance was done following the factorial design with the help of computer package Minitab 17.0. The data analysis was performed by F-test (Gomez & Gomez 1984). The mean of different treatment groups were compared using Fisher's least significant difference (LSD) test.

RESULTS

Effect of arbuscular mycorrhiza, cowdung and phosphorus

i. Macronutrient contents in red amaranth: In red amaranth, application of AM+CD+P showed maximum N, P, K, S and Mg content which was significantly higher than control and only application of AM, CD and P. The nitrogen content was found in AM + CD application was statistically similar to AM+CD+P. The combined application of CD+P and AM+P also increased nitrogen content significantly than the control condition. Application of AM+CD and AM+P increased P content statistically similar to AM+CD+P and AM+CD also increased P content significantly than control. The combined application of AM+CD, CD+P and AM+P increased potassium content significantly than control condition which was similar to T8. The effect of arbuscular mycorrhiza, cowdung and phosphorus on calcium content was statistically not significant. When AM was treated with phosphorus statistically similar Mg content was found compared to AM+CD+P.

ii. Micronutrient contents in red amaranth: The combined application of AM, CD and P showed maximum Fe, Mn, Cu and Zn content in red amaranth which was significantly higher than control. AM + P application showed statistically similar Fe and Zn content to AM+CD+P. The combined application of AM+CD, CD+P and AM+P also increased Cu contents significantly than control condition which was statistically similar to AM+CD+P.

iii. Macronutrient content in Indian spinach: The combined application of AM bio-fertilizer, cowdung and phosphorus showed maximum P, K, S, Ca and Mg content in Indian spinach which was significantly higher than control. Application of AM with phosphorus and AM with cowdung achieved the highest nitrogen content. Application of AM with phosphorus showed similar P and K content compared to AM+CD+P. Application of AM with CD or P also increased S and Mg content which was statistically similar to AM+CD+P.

iv. Micronutrient content in Indian spinach: The combined application of AM bio-fertilizer, cowdung and phosphorus showed maximum Fe, Mn, Cu and Zn content in Indian spinach which was significantly higher than control. The treatment CD + P achieved Fe content which was statistically similar to only CD and P application. Application of AM gave the statistically higher result to control. Application of phosphorus with cowdung produced Mn content which was statistically similar to AM + CD + P. The lowest Mn content was found in AM applied condition. Application of AM with cowdung and AM with phosphorus achieved Cu content which was statistically similar to AM + CD + P. The application of arbuscular mycorrhiza and phosphorus which was statistically similar with AM+CD+P.

v. Protein content: The results showed that protein content in Indian spinach was higher with phosphorus application than control treatment. Application of AM with phosphorus achieved 4.31% protein which was statistically similar to T_5 (CD + AM) and T_4 (P). In red amaranth, the maximum protein content was observed in AM, CD and P applied the treatment, which was statistically similar with AM+CD, CD+P and AM+P treatments.

Nutrient uptake by Red amaranth and Indian spinach and correlation among the nutrients

AM inoculation, organic matter and phosphorus supplementation increased N, P, K, S, Ca, Mg, Fe, Mn, Cu and Zn uptake. Always the maximum nutrient uptake was noticed in combined application of AM, CD & P. AM inoculation with CD or P also increased nutrient uptake than only CD or P application. Most of the nutrient contents were strongly correlated among themselves.

DISCUSSION

Our previous study showed that arbuscular mycorrhiza, cowdung and phosphorus enhanced growth and yield contributing characters of red amaranth and Indian spinach (Ghosh *et al.* 2017).

Application of AMF remarkably elevated the plant mineral nutrition, mainly with nitrogen and phosphorus (Salvioli *et al.* 2012, Colella *et al.* 2014). This dependence seems to be confirmed by results of our studies, in which application of AM supplemented with CD and P showed higher N and P content as compared to red amaranth and Indian spinach plants grown without AMF inoculation (Table 2 & 3). Guo *et al.* (2006) reported that in generally mycorrhizal colonization resulted in increased shoot N and P contents in onion. Arbuscular mycorrhizal fungus *Glomus intraradices* actively mobilize P from phosphates and increase the take-up of more in roots inoculated with *G. intraradices* than those un-inoculated (Antunes *et al.* 2007). In this study, the application of cowdung and phosphorus showed statistically identical phosphorus uptake. The results further indicated that arbuscular mycorrhiza biofertilizer, cowdung and phosphatic fertilizer treated pots performed better with respect to phosphorus uptake (Table 2 & 3). Similarly, Harrison *et al.* (2010) reported that phosphorus was often the key element for increased growth or fitness of mycorrhizal plants because it was transported in hyphae in large amounts compared to the plant phosphorus demand. Moreover, (Kehri & Chandra 2001) suggested that it was possible to reduce the phosphate recommendation for black gram with inoculation of effective AM fungi.

Table 2. Effect of arbuscular mycorrhiza (AM), cowdung (CD) and phosphorus (P) on nutrients content of red amaranth.

Treatments	N (%)	P (%)	K (%)	S (%)	Ca (%)	Mg (%)	Fe (μg g^{-1})	Mn (μg g^{-1})	Cu (μg g^{-1})	Zn (μg g^{-1})	Protein (%)
T_1: Control	0.63g	0.22d	0.23c	0.07d	0.31	0.35d	4.08e	3.32e	1.74b	2.60c	3.96d
T_2: AM	0.82f	0.22d	0.28b	0.07d	0.33	0.43c	5.60c	4.00d	2.02b	3.00bc	5.13c
T_3: CD	0.87e	0.39c	0.28b	0.11c	0.32	0.45c	6.36b	3.20e	2.03b	2.60c	5.41c
T_4: P	0.94d	0.47b	0.27b	0.12c	0.33	0.37d	6.12b	3.80e	2.53a	2.80bc	5.89bc
T_5: AM+CD	1.09a	0.39c	0.33a	0.12c	0.33	0.44c	6.42b	5.40e	2.59a	3.20b	6.83a
T_6: CD+P	1.01c	0.52a	0.33a	0.13c	0.33	0.52b	5.18d	6.40b	2.80a	3.00bc	6.33ab
T_7: AM+P	1.05b	0.49ab	0.35a	0.17b	0.34	0.55ab	6.50ab	5.52c	2.96a	3.80a	6.56ab
T_8: AM+CD+P	1.09a	0.53a	0.34a	0.23a	0.34	0.59a	6.88a	7.60a	2.95a	4.00a	6.80a
LSD (0.05)	0.012	0.04	0.027	0.022	0.022	0.040	0.39	0.67	0.49	0.57	0.87
CV (%)	16.47	14.27	17.65	10.27	16.48	15.15	7.21	10.91	17.45	15.59	6.59
Level of sig.	**	**	*	*	NS	**	**	**	**	**	**

Note: * Significant at 5% and ** significant at 1% level of probability.
In a column, the figure(s) having the same letter are not significantly different at 5% or 1% level of probability by DMRT.

Table 3. Effect of arbuscular mycorrhiza (AM), cowdung (CD) and phosphorus (P) on nutrients content of Indian spinach.

Treatments	N (%)	P (%)	K (%)	S (%)	Ca (%)	Mg (%)	Fe (μg g^{-1})	Mn (μg g^{-1})	Cu (μg g^{-1})	Zn (μg g^{-1})	Protein (%)
T_1: Control	0.53e	0.31d	0.57c	0.10b	0.40e	0.40c	3.44f	1.12d	2.52f	6.13f	3.30f
T_2: AM	0.59c	0.43bc	0.61bc	0.10b	0.43de	0.41bc	4.20e	1.12d	2.63f	6.24ef	3.66de
T_3: CD	0.61cd	0.37cd	0.60bc	0.12ab	0.45d	0.40c	4.62c	1.32b	3.18bcd	6.44d	3.84cd
T_4: P	0.66ab	0.40c	0.59bc	0.12ab	0.50bc	0.41bc	4.32d	1.22c	2.97e	6.42ed	4.13ab
T_5: AM+CD	0.69a	0.40c	0.62abc	0.13ab	0.47cd	0.44ab	4.90b	1.32b	3.29bc	7.03c	4.33a
T_6: CD+P	0.57d	0.48b	0.60bc	0.14a	0.52ab	0.41bc	4.66c	1.34b	3.17cd	7.25b	3.54e
T_7: AM+P	0.69a	0.63a	0.64ab	0.14a	0.55a	0.45a	4.48c	1.30b	3.30b	7.61a	4.31a
T_8: AM+CD+P	0.63bc	0.66a	0.66a	0.14a	0.55a	0.46a	5.36a	1.38a	3.48a	7.60a	3.96bc
LSD (0.05)	0.04	0.07	0.05	0.03	0.04	0.03	0.184	0.041	0.12	0.18	0.23
CV (%)	15.73	10.75	13.76	9.25	19.91	15.78	6.77	10.23	7.85	4.48	6.35
Level of sig.	**	**	**	*	**	**	**	**	**	**	**

Note: * Significant at 5% and ** significant at 1% level of probability.
In a column, the figure(s) having the same letter are not significantly different at 5% or 1% level of probability by DMRT.

In the opinion of Candido *et al.* (2015), cooperation with mycorrhizal mycelium also improves the nutrition of a host plant with potassium. In the present study, the significant influence of mycorrhization on improved potassium supply was confirmed for red amaranth and Indian spinach was grown on cowdung and phosphorus supplemented condition (Table 2 & 3). A similar result was found by Giri *et al.* (2005) and Zuccarini (2007), who observed that mycorrhizal symbiosis stimulated K content of *Cassia siamea* and lettuce, respectively.

When only AM fungi were inoculated no change in S content was noticed in both vegetables. But when AM fungi applied with cowdung or phosphorus or with both, the S content increased in both vegetables. It is clear from the present study that, solo application of AM fungi was less effective than a combined application with cowdung or phosphorus (Table 2 & 3). This result was totally different to those of (Allen & Shachar-Hill 2009) and (Azcón-Aguilar *et al.* 2003), who described that AMF enhanced S content in carrot and lettuce, respectively. Further study on red amaranth and Indian spinach are required to clarify this issue. AM inoculation with organic matter and phosphorus also increased Mg content in both vegetables and a similar result was found by Azcón-Aguilar *et al.* (2003).

Our study revealed a non-significant effect of AM fungi on Ca contents compared to non-colonized red amaranth plants (Table 2). Similarly, Kothari *et al.* (1990) found a decreased concentration of Ca in the shoot of maize under AMF inoculated condition. Several previous studies showed inconsistent results regarding Ca contents in different species due to mycorrhizal inoculation (Clark 1997, Alloush *et al.* 2000, Bagayoko *et al.* 2000). AMF associated plants usually maintain relatively low Ca content because the Ca-loaded polyphosphates might hamper the functioning of AM fungi (Marschner & Dell 1994). Li *et al.* (2007) observed that inoculation with AM fungi significantly increased the contents of calcium in taro. On the contrary, calcium content increased in Indian spinach due to the application of AM supplemented with cowdung and phosphorus (Table 3). A similar result was found by (Rosen *et al.* 1994), who observed that the calcium content in spinach beets significantly increased by municipal solid waste over chemical fertilizer.

In the current study, we found a positive impact of AMF inoculation on the Fe, Mn, Cu and Zn contents in red amaranth (Table 4). Our result is in line with some previous studies (Hirata *et al.* 1988, Gavito *et al.* 2000). In other experiments, similar results were found where the application of AM fungi increase Fe (Farzaneh *et al.* 2011), Mn (Farzaneh *et al.* 2011), Cu (Giri *et al.* 2005) and Zn (Ortas & Akpinar 2006) content in different plant shoots. In Indian spinach, AMF inoculation with CD or P or both increased Fe, Cu and Zn content (Table 5). They are in line with (Bi *et al.* 2003) and (Nogueira *et al.* 2007). (Al-Karaki 2006) also observed that shoot contents of Fe were higher in AM compared with non-AM tomato plants grown under non-saline and saline water conditions. On the other hand, application of AM solely or combined with CD did not increase Mn content, but when AM applied with P than Mn content enhanced (Table 4 & 5). A totally different result was found by (Farzaneh *et al.* 2011), who stated that AMF colonization resulted in a significantly higher Mn concentration in chickpea. Detailed studies regarding this issue are necessary for a broad understanding of this process.

Table 4. Correlation matrix among nutrient contents of red amaranth.

Characters	Correlation coefficient (r value)									
	Fresh weight	**N**	**P**	**K**	**S**	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**
N	0.609**									
P	0.544**	0.799**								
K	0.572**	0.811**	0.639**							
S	0.427**	0.737**	0.791**	0.687**						
Ca	0.580**	0.510**	0.384*	0.549**	0.465**					
Mg	0.632**	0.651**	0.625**	0.739**	0.795**	0.477**				
Fe	0.345*	0.755**	0.583**	0.567**	0.641**	0.396**	0.537**			
Mn	0.568**	0.627**	0.590**	0.629**	0.702**	0.269^{NS}	0.566**	0.280^{NS}		
Cu	0.214^{NS}	0.832**	0.797**	0.694**	0.759**	0.587**	0.696**	0.573**	0.614**	
Zn	0.300^{NS}	0.364*	0.302^{NS}	0.442**	0.514**	0.238^{NS}	0.348*	0.358*	0.551**	0.438**

Note: ** Significant at 1% and * Significant at 5% level of probability, NS = Not significant.
Tabulated value of r with 38 df are 0.404 at 1% level and 0.313 at 5 % level.

Table 5. Correlation matrix among nutrient contents of Indian spinach.

Characters	Correlation coefficient (r value)									
	Fresh weight	**N**	**P**	**K**	**S**	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**
N	0.630**									
P	0.662**	0.378*								
K	0.501**	0.368*	0.529**							
S	0.507**	0.398*	0.399**	0.221^{NS}						
Ca	0.706**	0.434**	0.712**	0.297^{NS}	0.632**					
Mg	0.501**	0.269^{NS}	0.606**	0.342*	0.300*	0.500**				
Fe	0.814**	0.506**	0.562**	0.546**	0.385**	0.504**	0.415**			
Mn	0.755**	0.482**	0.502**	0.277^{NS}	0.614**	0.637**	0.379*	0.738**		
Cu	0.772**	0.522**	0.503**	0.435**	0.402**	0.636**	0.507**	0.711**	0.767**	
Zn	0.741**	0.458**	0.785**	0.541**	0.537**	0.768**	0.672**	0.645**	0.705**	0.745**

Note: ** Significant at 1% and * Significant at 5% level of probability, NS = Not significant.
Tabulated value of r with 38 df are 0.404 at 1% level and 0.313 at 5 % level.

The present investigation showed that AM-inoculated red amaranth and Indian spinach plants had higher contents of protein than nonmycorrhizal plants (Table 4 & 5). The observed increase in protein contents in mycorrhizal plants is in a good conformity with the results of other researchers (Abdel-Fattah *et al.* 2014). Again, in our research, always the protein content in both the vegetables was higher when phosphorus was applied with AM fungi (Table 1 & 2). This may demonstrate the role of phosphorus in the regulation of symbiosis through synthesis, simulation, or activation of proteins and enzymes (Lambais & Mehdy 1995). However, Epstein & Bloom (1999) emphasizes that phosphorus has a direct effect on diverse enzymatic plant processes, very often acting as a biochemical co-factor. This protein increment leads to membrane stabilization and helps plants to grow and develop under adverse conditions (Goudarzi & Pakniyat 2009).

AMF are capable of up taking and transporting almost all the 15 essential macro- and micronutrients. AMF mobilize firm or rock-bound nutrients such as phosphorous, iron, and other tightly arrested mineral nutrients in the soil (Teotia *et al*. 2017). In the present study, strong mycorrhizal effects on red amaranth and Indian spinach were also observed when looking at the nutrient uptake. We found an increase of N, P, K, S, Mg, Fe, Mn, Zn, and Cu uptake after AMF inoculation. Only changes in Ca uptakes with AM-red amaranth were relatively small (Table 6 & 7). Similar positive effects of AMF on nutrient uptake in legumes were reported earlier (Ilbas & Sahin 2005, Lin *et al.* 2007). Our results are also in accordance with those of previous work on chickpea by Akhtar & Siddiqui (2006), showing increased P and K uptake in mycorrhizal plants. AMF produces an array of siderophores and act as chelating agents that ultimately increase the nutrient uptake and transport in plants (Caris *et al.* 1998). In higher plants the AMF association also helps plant by increasing the surface area of the root by penetrating their hyphae deep into the soil (Schnepf *et al.* 2011). AMF interactions also may influence plant growth through the improvement in nutritional attainment by mobilizing nutrient from the organic substrates, by improving the fertilizer use efficacy, or by an advantageous alliance with other soil microbes (Finlay 2008).

Table 6. Effect of AM, cowdung and phosphorus on macro- and micronutrients uptake (per plant) of red amaranth.

Treatments	N (mg)	P (mg)	K (mg)	S (mg)	Ca (mg)	Mg (mg)	Fe (µg)	Mn (µg)	Cu (µg)	Zn (µg)
T_1: Control	1.10e	0.36c	0.40e	0.10e	0.54c	0.58d	0.007f	0.006e	0.003d	0.005g
T_2: AM	6.44d	1.76c	2.24d	0.56d	2.56b	3.40c	0.045e	0.030d	0.016c	0.026de
T_3: CD	8.26bcd	3.80b	2.74cd	1.00cd	3.04b	4.30b	0.061bc	0.031d	0.019c	0.025ef
T_4: P	7.46c	3.80b	2.10d	0.90cd	2.64b	3.04c	0.049de	0.026d	0.020c	0.019f
T_5: AM+CD	11.7b	4.14b	3.58bc	1.24c	3.58b	4.68bc	0.068b	0.056c	0.027b	0.034c
T_6: CD+P	10.7bc	5.42b	3.48bc	1.42bc	3.54b	5.50b	0.055cd	0.067b	0.030b	0.032cd
T_7: AM+P	11.8b	5.50b	3.96b	1.88b	3.86b	6.20b	0.073b	0.061bc	0.033b	0.042b
T_8: AM+CD+P	19.7a	9.58a	6.14a	4.16a	6.18a	10.6a	0.124a	0.135a	0.053a	0.069a
LSD (0.05)	4.14	1.81	1.21	0.59	1.35	2.02	0.006	0.006	0.006	0.006
CV (%)	9.97	15.77	17.29	11.48	16.30	14.63	13.29	18.22	13.05	15.14
Level of sig.	**	**	**	**	**	**	**	**	**	**

Note: ** = Significant at 1% level of probability.
In a column, the figure(s) having the same letter are not significantly different at 1% level of probability by DMRT.

Table 7. Effect of AM, cowdung and phosphorus on macro- and micronutrients uptake (per plant) of Indian spinach.

Treatments	N (mg)	P (mg)	K (mg)	S (mg)	Ca (mg)	Mg (mg)	Fe (µg)	Mn (µg)	Cu (µg)	Zn (µg)
T_1: Control	4.86d	2.90e	5.22e	0.94d	3.78e	3.70e	0.03e	0.01c	0.02e	0.06e
T_2: AM	12.3c	9.00d	12.8d	2.16c	8.98d	8.62d	0.09d	0.02bc	0.06d	0.13d
T_3: CD	12.6c	7.62d	12.4d	2.54b	9.24d	8.26d	0.09d	0.03b	0.07cd	0.13d
T_4: P	13.8c	8.34d	12.2d	2.60b	10.4cd	8.46d	0.09d	0.03b	0.06d	0.13d
T_5: AM+CD	16.3b	9.48cd	14.7c	2.98b	11.0bc	10.3c	0.12c	0.03b	0.08c	0.17c
T_6: CD+P	13.6c	11.5c	14.3c	3.28b	12.4b	9.88c	0.11c	0.03b	0.08c	0.17c
T_7: AM+P	24.9a	22.8b	23.0b	5.22a	19.9a	16.1b	0.16b	0.05a	0.12b	0.27b
T_8: AM+CD+P	24.9a	25.8a	26.1a	5.46a	21.6a	18.0a	0.21a	0.05a	0.14a	0.30a
LSD (0.05)	1.77	2.17	1.67	0.75	1.67	1.32	0.006	0.006	0.006	0.006
CV (%)	6.57	9.02	6.64	15.65	7.80	8.04	19.72	16.07	16.85	14.60
Level of sig.	**	**	**	**	**	**	**	**	**	**

Note: ** = Significant at 1% level of probability.
In a column, the figure(s) having the same letter are not significantly different at 1% level of probability by DMRT.

CONCLUSIONS

In summary, the capability of AMF to increase mineral nutrition in both the plant species is clearly positively influenced. AMF could be used for the production mineral rich leafy vegetables to provide almost all the essential minerals and organic nutrients at a higher quantity to humans for maintenance of health. On the other side, mycorrhizal fungi not merely assist in the uptake of the major plant nutrients such as P, K and N but also help in captivating other micronutrients like Fe, Cu, Zn, Mn, etc. Consequently, mycorrhiza is established as a significant association for nutrient management in the ecosystem.

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