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ARBUSCULAR MYCORRHIZAE UNDER $CUSO_4$ STRESS COMMUNITY STRUCTURE OF ARBUSCULAR MYCORRHIZAE UNDER $CUSO_4$ STRESS IN *CAPSICUM ANNUUM* L. & *ZEA MAYS* L.

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Abstract

Community structure and ecology of arbuscular mycorrhizal fungi was studied in the present study using two host plant species i.e. *Capsicum annuum* L. and *Zea mays* L. under $CuSO_4$ stress. Five levels of copper sulfate ($CuSO_4$) i.e. 0 ppm (control), 25 ppm, 50 ppm, 75 ppm, and 100 ppm were used to elucidate their influence on mycorrhizal community. Results showed that some spores disappeared with increased metal content while other spores were abundant even at a high level of stress. Present studies supported the stress tolerance mechanism conferred by AMF spore density and diversity. Value of Simpson index was shown to decrease from 3.58 to 2.42. Shannon index value was changed from 0.27 to 0.51. Similar rise in the values was observed for spore diversity i.e. 20.94 to 79.13. However, it may be concluded that spore ecotypes might vary in their abundance depending upon the host plant and soil physical-chemical characters that control the metal availability to plants. Among all the four plant varieties, ghotki chilli seemed to have less species associated with it. It can be concluded that when compared to the control, more mycorrhizal types were found to be associated with plants under stress which could prove the tolerance of mycorrhizae against the heavy metals and their positive role in protecting plant from the toxicity of heavy metals.

Key words: *Capsicum annuum* L.; *Zea mays* L.; Metal stress; Arbuscular mycorrhizal community structure (AMF).

Introduction

"Mycorrhizae", literally means "fungus roots" which was coined by A.B. Frank (1885) specifically for mutualistic symbiosis mostly present in fungi and underground plants parts (Silva & Uchida, 2000). This association involves two partners i.e. vascular plants and soil fungi, in which both get benefited by exchanging nutrients and energy for development (Hodge, 2000). Mycorrhizal symbiosis is significant for plant nutrition, plant growth, development, and soil quality (Jeffries *et al.*, 2003).

Many investigations show that mycorrhizal associations may contribute to provide resistance against chemicals substances. Mycelium is endurable to many chemical substances including pesticides (endrin, chlordane, methonylcorbofuran), herbicides (glyphosphate, fuazifobutyl) and other chemical agents. As chemical substances tend to be stored and immobilized into the mycorrhizal sheath therefore it act as barrier from high concentration of toxic heavy metals (Chhabra & Jalali, 2013).

Community structure of AMF (Arbuscular Mycorrhizal Fungi) determines the ecology of plants and their distribution and abundance through the landscape (Tilman, 1982). It also controls the interaction between plants and environment, (Brown, 1990) and plant response against biotic stress i.e. pathogens (Dobson & Crawley, 1994) and abiotic stress i.e. drought, heavy metal, pesticide.

Trace elements are required in minimum quantity to maintain plant body and its physiology. These trace elements include copper, zinc, iron (essential) etc, other non-essential elements including cadmium, lead, and mercury. Excess of these elements available in soil may prove hazardous for plant life (Hall & Williams, 2003). Source of these heavy metals may include mining, use of pesticides and herbicides in agriculture, and military practices.

As mycorrhizae are present in terrestrial ecosystems and play role in the nutrients transportation. Mycorrhizae have the ability to uptake the heavy metals and transport them to plants. However, mantle provide physical barrier against uptake of heavy metal (Khan, 2005). AMF has phytoremediation ability thus improving plant growth under heavy metal stress (Yanga, 2015).

Phylum glomeromycota is the most abundant group of mycorrhizal fungi (Schussler *et al.*, 2001). It establishes its network with host plants. There are some spores that do not have ability to tolerate heavy metal stress in un-contaminated soils only. However, the spores of polluted soils have the ability to tolerate the heavy metal stress. Tolerance against pollution or heavy metal uptake is enhanced by the spores after one or two generations (Shalaby, 2003). Further studies showed that tolerant spores rapidly colonize the plant species under stress conditions although their number is low.

The objective of this study was to investigate species density and diversity of various inoculums associated with *Zea mays* L. and *Capsicum annum* L. under $CuSO_4$ stress picturing the best community structure of Arbuscular Mycorrhiza in local soils under metal stress and their utility against metal tolerance.

Materials and Method

Study was conducted to investigate the community structure of arbuscular mycorrhizae under $CuSO_4$ stress in two plants viz. *Capsicum annuum* (Ghotki chilli & Sanam chilli, the two verities used) and *Zea mays* (Desi maize & Javen maize). Experiment was conducted with 2 main factors i.e. two plants along with their varieties and five $CuSO_4$ concentrations in triplicate manner. Soil,

used as a source of inoculum, was obtained from a local plant nursery. Seeds were obtained from seed corporation Bahawalpur. Seeds were kept in petri dish filled with water for one night. Sixty pots of uniform size were filled with soil. Pots were watered before sowing. Seeds were sown in March, 2015. Treatment was started when seedlings were one week older. $CuSO_4$ was given in 4 different concentrations i.e. 25 ppm, 50 ppm, 100 ppm against control treatment (0 ppm). Unit used for metal treatment application was Parts per Million (ppm). Treatment was given for eight weeks at regular interval of two weeks.

Roots were harvested after two months. Roots and rhizospheric soil samples were taken from all the sixty pots. Roots and soil samples were collected in polythene bag, properly labeled and transported to Mycology laboratory of Life Sciences department of The Islamia University of Bahawalpur. Roots were washed properly and fixed in FAA (Formaldehyde, Acetic Acid, Alcohol, 5:5:90). Roots were processed using the method of Phillips & Hayman (1970) modified by Chaudhary *et al*. (2012). Biermann & Lindermann's (1981) method was used to measure the infection percentage.

500 g of collected rhizospheric soil was sent to Soil and Water Testing Laboratory, Bahawalpur for Physico-chemical analysis. Physico-chemical analysis of rhizospheric soil collected from all soils is given in Table 1. Wet sieving and decanting technique of Gerdemann & Nicolson (1963) was used to isolate AMF spores from rhizospheric soil. 10-30 spores were picked up using tooth pick and mounted in PVLG and Melzer's reagent. Slight pressure was applied to remove extra stain and to clarify spore walls for their easy identification. Spores were observed under Labomed Digi-2 Compound microscope. Spores were identified on the basis of morphological characteristics of AMF spores using the identification manuals of Schenck & Perez (1990) and International collection of Vesicular Arbuscular Mycorrhizal fungi (http://invam.caf.wvu.edu). Spore density, diversity index and frequency of occurrence were recorded. The descriptive statistics of mycorrhizal colonization and AMF spore distribution was done using MSTAT C and SPSS 16.0. Pearson correlation among soil physico-chemical characteristics was done using SPSS 16.0. Analysis of Variance (ANOVA) and Least Significant Difference (LSD) was measured using MSTAT C. Shannon-Weiner Diversity Index of AMF diversity and Simpson Index of Species Dominance were computed manually.

Results

Percent root colonization was recorded in all plants under five $CuSO_4$ treatments. Hyphal percentage is shown in Fig. 1. Maximum hyphal colonization was seen in Javen variety of maize treated with 25 ppm. Minimum percentage of hyphal colonization was observed in Sanam chilli treated with 50 ppm $CuSO_4$ (T3). Hyphal colonization was highest in control. It showed decreasing trend with increased metal content except in 75 ppm treatment where its value was seen to be highest than the rest of treatments.

Fig. 2 shows vesicular colonization percentage. Maximum percentage of vesicles were observed in roots of Javen maize treated with 75 ppm. Vesicular colonization percentage was shown to be decreased with increasing metal concentration. This was true for all concentrations except 75 ppm. Minimum value of vesicles was seen in Javen maize treated with 50 ppm.

Arbuscular colonization percentage is shown in Fig. 3. The highest percentage of arbuscules was found at 25 ppm and it was found to be low at 100 ppm. Maximum number of arbuscules were observed in Desi maize when treated with 50 ppm. Minimum value was observed in roots of Sanam chilli treated with 0 and 50 ppm.

Intra-radical spore colonization is shown in Fig. 4. Maximum spores were present in the roots of Javen maize treated with 100 ppm $CuSO_4$. Whereas, minimum value of spores percentage was seen in 50 ppm and 100 ppm treatment in roots of Sanam Chilli. Spore percentage was decreased at 50 ppm. Overall, spore colonization seemed to be fluctuating with the increase of the metal content.

Analysis of variance of root colonization under $CuSO_4$ stress in response to host plant varieties and metal stress was calculated (Table 2). Concentrations and plant varieties, both had significant effect on hyphal percentage, arbuscular percentage, intra-radical spore percentage and vesicular percentage. Combined effect of both factors significantly improved AMF colonization percentage.

Table 3 shows Pearson's correlation coefficients among soil physico-chemical soil parameters and AMF root colonization. Electrical conductivity (EC) showed a positive correlation with organic matter, vesicular colonization ($p<0.05$) and available potassium ($p<0.01$) but a strong negative correlation with available phosphorus ($p<0.01$). Organic matter showed strong correlation with saturation percentage ($p<0.01$) and available potassium content ($p<0.05$) but strong negative correlation with available phosphorus ($p<0.01$). Available phosphorus showed strong negative correlation with available potassium ($p<0.01$). Hyphal colonization showed strong negative correlation with arbuscular colonization ($p<0.01$). Vesicular colonization correlated positively with arbuscular colonization ($p<0.05$).

Table 1. Physico-chemical analysis of rhizospheric soil sampled from all pots.

Plant	Varieties	EC (dSm^{-1})	pH	O.M. (%)	P (ppm)	K (ppm)	S.P. (%)
Zea mays	Desi Maize	1.55 ± 0.13	7.85 ± 0.31	0.43 ± 0.08	16.14 ± 1.259	59.93 ± 6.32	41.7 ± 0.645
	Javen Maize	1.27 ± 0.10	7.75 ± 0.15	0.52 ± 0.08	13.32 ± 1.181	60.80 ± 3.013	42.2 ± 1.196
Capsicum annuum	SanamChilli	3.51 ± 0.41	7.77 ± 0.17	0.71 ± 0.04	6.72 ± 0.272	137.65 ± 8.76	43.53 ± 0.86
	GhotkiChilli	4.06 ± 0.56	8.0 ± 0.29	0.67 ± 0.02	7.37 ± 0.441	135.8 ± 9.95	42.4 ± 0.49

Legend: EC = Electrical Conductivity, pH = Negative Log of Hydrogen Ion, O. M. = Organic Matter, P = Available Phosphorus Content, K = Potassium, S. P. %= Saturation Percentage. (Mean ± Standard Error)

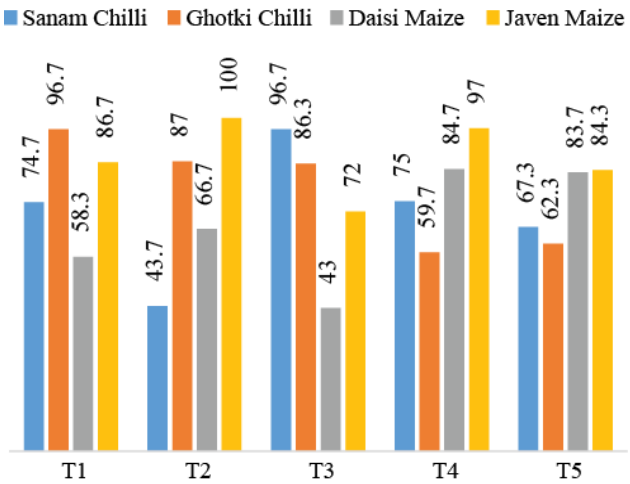

Fig. 1. Comparison of Hyphal Percentage among soils of all varieties of *Zea mays* and *Capsicum annum*.

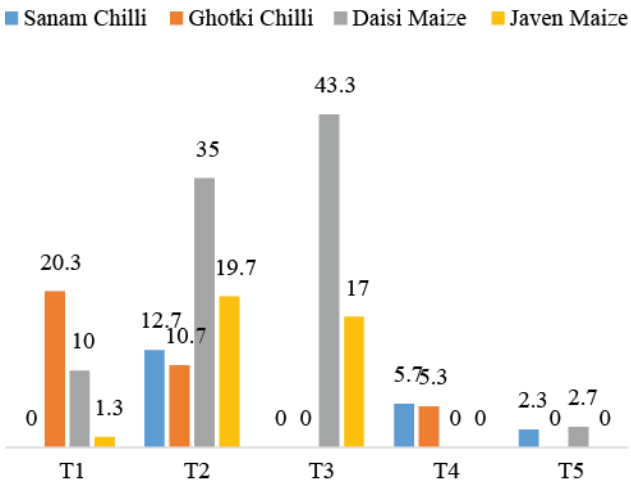

Fig. 3. Comparison of Arbuscules Percentage among soils of all varieties of *Zea mays* and *Capsicum annum*.

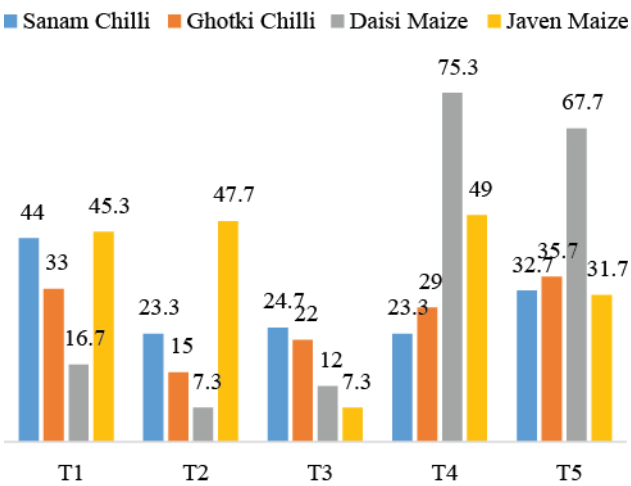

Fig. 2. Comparison of Vesicular Percentage among soils of all varieties of *Zea mays* and *Capsicum annum*.

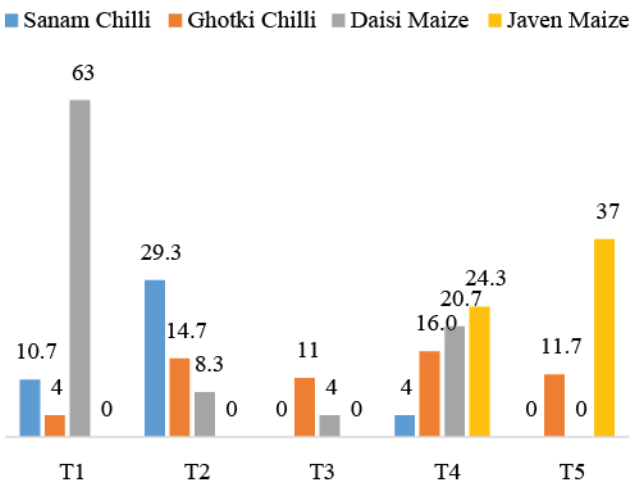

Fig. 4. Comparison of Intra-radical Spore Percentage among soils of all varieties of *Zea mays* and *Capsicum annum*.

Table 2. Analysis of Variance of Root Colonization under $CuSO_4$ stress.

Source of variation	Hyphal colonization	Vesicular colonization	Intra-radical spores colonization	Arbuscular colonization
Treatment	78.44**	1707.61**	413.93**	583.23**
Plant (varieties)	1231.31**	317.2**	294.76**	377.39**
Treatment × Plant (varieties)	1039.93**	970.19**	996.26**	321.48**

** Correlation is significant at 0.01 alpha level

Table 3. Pearson's Correlation coefficients among soil physico-chemical soil parameters and AMF root colonization.

	Electrical conductivity	pH	Organic matter	Available phosphorus	Available potassium	Saturation percentage	Hyphal colonization	Vesicular colonization	Arbuscular colonization
pH	-0.02								
Organic matter	**0.28***	0.00							
Available phosphorus	**-0.47****	-0.03	**-0.63****						
Available potassium	**0.75****	-0.02	**0.32***	**-0.64****					
Saturation percentage	0.56	0.17	**0.35****	-0.20	0.17				
Hyphal colonization	-0.11	-0.08	-0.03	0.08	-0.10	-0.07			
Vesicular colonization	**0.33***	-0.14	0.03	0.09	0.18	-0.17	0.20		
Arbuscular colonization	-0.04	0.06	-0.24	0.12	-0.14	-0.16	**-0.15***	**0.03***	
Intra-radical spore colonization	-0.19	-0.02	-0.08	0.06	-0.16	0.12	-0.14	-0.30	-0.02

*Correlation is significant at 0.05 level (2–tailed)
**Correlation is significant at 0.01 level (2–tailed)

Table 4. AMF species diversity indices and spore density for $CuSO_4$ treatments of all plants.

Diversity Indices / Density	Varieties	Before treatment	After treatment
Shannon –Weiner Index	Sanam Chilli	0.07	0.15
	Ghotki Chilli	0.08	0.06
	Desi Maize	0.07	0.15
	Javen Maize	0.05	0.15
		0.27	0.51
Simpson Index	Sanam Chilli	0.90	0.54
	Ghotki Chilli	0.87	0.90
	Desi Maize	0.89	0.51
	Javen Maize	0.92	0.46
		3.58	2.42
Spore Density	Sanam Chilli	5.08	23.02
	Ghotki Chilli	6.47	4.78
	Desi Maize	5.49	24.43
	Javen Maize	3.90	26.90
		20.94	79.13

AMF species diversity can be explained in terms of diversity indices (Table 4). Highest value for Shannon-Weiner index was shown by Sanam Chilli, Desi and Javen maize after applying treatment. Before treatment, highest index was shown by Ghotki Chilli followed by Sanam chilli and Ghotki maize, both had the same value. Lowest value was shown by Javen maize. Overall value of Shannon-Weiner index increased after applying $CuSO_4$. Another index used for AMF diversity assessment is Simpson diversity index. Before treatment, highest value was given by Javen maize followed by Sanam chilli, then Desi maize, and Ghotki chilli. These values of Simpson index were seen to be influenced by $CuSO_4$ concentrations. Ghotki chilli showed the highest diversity index followed by Sanam chilli, Desi maize and Javen maize. Overall value of Simpson diversity index was decreased after applying $CuSO_4$.

Table 4 showed the analyses of the spore density among four plant varieties before and after treatment to have a comprehensive understanding of $CuSO_4$ effect that it has caused on community structure of AMF. It can be clearly observed that spore density associated with Sanam chilli was quite low before treatment but was shown to increase after applying stress. Similar results were observed for other studied varieties of maize that included Javen maize and Desi maize. For Ghotki chilli, relative density was found to be decreased with increase in concentration of applied stress.

Discussion

Metal content in soil, soil physico-chemical properties and host plant species are the major factors structuring the colonization status of AMF communities. Percentage of hyphae, intra-radical spore, arbuscules, and vesicles varied significantly among all treatments. Overall mycorrhizal root infectivity was found to be decreased with the increased metal contamination. However, some of parameters like vesicular colonization found to be increased. Other parameters including root sporulation percentage and arbuscular colonization percentage were found to be decreased.

Variation in root colonization not only occur in relation to metal concentration but also show their relation with plant varieties. So, all of the four varieties under study did not behave in similar manner. Even the two varieties of same species showed variability in their response towards metal stress. Difference in AMF colonization percentage can be attributed to better adaptation, increased $CuSO_4$ tolerance and compatibility with these varieties. Similar variation in root colonization of AMF has been reported by Valarini *et al*. (2009) and Cornejo *et al*. (2007). Plant can avoid heavy metal toxicity but this ability varies from plant species to species, type of metal, its concentration, and mycorrhizae fungal type (Cicatelli *et al*., 2010).

Hyphal colonization percentage showed decreasing pattern with increasing levels of metal content that also inhibited the spore presence inside the root. Our results were found to be consistent with that of Pawlowska & Charvat (2004) along with Tyler *et al*. (1980). Increase in vesicular colonization demonstrated their role against accumulated metal. Similar results were purposed by Chanda *et al*. (2014) who also observed increased number of vesicles with increased stress levels. Hart & Reader (2005) suggested that spore inoculums significantly differ in forming external mycelium so they may differ in root colonization pattern.

In this investigation, root colonization is apparently correlated with sporulation; however, these processes are not necessarily dependent on each other and previously published data are ambiguous (Abbott & Robson, 1991; Douds, 1994). It has been observed that intraradical development of spores influence both the beginning of sporulation as well as the production of external hyphae (Gazey *et al*., 1992). Clapp *et al*. (1995) under field condition demonstrated a positive relationship between the spore distribution and root colonization. More detailed work carried out under controlled conditions suggests that spore production is related more strongly to the length of root colonization than to the percent root colonization (Douds & Schenck, 1990; Gazey *et al*., 1992). Other papers showed that plants with finer roots favored the sporulation of AMF (Kormanick *et al*., 1980, and yet others correlated

the increased number of spores with the improved nutritional state of the plants (Louis & Lim, 1987).

AMF colonization status varies widely depending upon physico-chemical characters of soil. Phosphorus is the major factor affecting the mycorrhizal colonization status. Mycorrhizal colonization is low when phosphorus is available to plants. Under low availability of phosphorus, colonization of mycorrhizae show tremendous increase in terms of spore abundance and root colonization (Sunilkumar & Garampalli, 2010).

pH is an important factor that influence the spore abundance and phosphorus availability (Michel-Rosales & Valdes, 1996). For the growth of *Zea mays* L. and *Capsicum annum* L. pH should range between 5-7 (Landon, 1991). However, the pH of studied soil recorded was slightly basic. Soil pH ranged from 7.6 to 8.1 in all soils either of control or those treated with metals.

Phosphorus availability is maximum when soil pH ranges from 6.0 to 7.0. Phosphorus availability reduces at alkaline pH (Wang *et al*., 2010). In present study, overall pH was alkaline and phosphorus availability was quite low. But there was no significant correlation between pH and phosphorus availability. However, researchers confirm the ability of arbuscular mycorrhizal fungi (AMF) to take up more metal from alkaline soil. However, contradictory results were given by Pawlowska *et al*. (2000). Wang *et al*. (1993) observed negative correlation between soil pH and mycorrhizal colonization. Others also confirmed that AMF colonization was significantly sensitive to even slight change in pH (Angle & Heckman, 1986).

Results showed less availability of organic content and phosphorus content. Alkaline soils have high potassium contents (Kim, 1994). Organic matter has the ability to retain metals in a form to make them less available to plants. Low amount of organic matter will allow metals to get absorbed by the plant. Similar inverse relation was discussed by Kushwaha *et al*. (2016). In present study, $CuSO_4$ was applied externally with amount increasing with each increment in treatment level, amount of organic matter remained unchanged. This low amount encountered only small amount of metal making it still available to plant as a stressor leading to the establishment of mycorrhizal association (Del Castilho *et al*., 1993).

Electrical conductivity showed positive correlation (p<0.05) with vesicular colonization. The value of electrical conductivity showed slight increase as the metal content was increased. Electrical conductivity is known to play an important role in plant growth. In present study, electrical conductivity improved mycorrhizal colonization status with increase in metal content. Similar results were obtained by Cseresnyes *et al*. (2013). Cseresnyes *et al*. (2014) also suggested the positive correlation of electrical conductivity and mycorrhizal colonization with metal concentration.

Saturation percentage showed no positive or significant relation with mycorrhizal colonization in this study. However, soil saturation play an important role in plant growth. Low moisture content may lead to drought stress and establishment of mycorrhizal community. Contradictory results were given by Ruiz-Sanchez *et al*. (2010) who considered moisture as an essential component to determine the mycorrhizal status. Smith *et al*. (2010) observed positive correlation between mycorrhizal colonization and available moisture content.

Conclusion

Present study determines that AMF colonization varies depending upon metal content, plant varieties and soil physico-chemical properties. Spore density and AMF root colonization were recorded to increase with metal content in all varieties except desi maize where spore density was reduced. General concept is that spore density and diversity increase with metal content. However, this is not the case in present research. Spore number was increased in all varieties of *Zea mays* L. and *Capsicum annuum* L. except Desi maize with metal content supporting the concept that not all the morphotypes are metal tolerant. Metal tolerance lead to the establishment of morphotypes that were previously absent or low content in control treated soil. Mycorrhizal community depends on plant age and soil pH value. Also the tolerant morphotypes have genotype unique to non-tolerant morphotypes. In present study, Javen maize represent it self as a metal tolerant variety having maximum spore density and species richness associated with it. While Ghotki chilli showed less spore density and species richness associated with it.

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