J. Phytol. Res. **33**(1):1- 11, 2020 **ISSN 0970-5767**

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH TOLERANCE OF WHEAT (*TRITICUM AESTIVUM* L.)TO METALS FROM ENGINEERING INDUSTRY.

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The study was performed to observe the effects of Arbuscular Mycorrhizal fungi.*Glomus hoi* and metals on growth parameters of wheat (*Triticumaestivum* L.) variety Raj4238underpot trials.There were two sets of experimentto study effect of Iron and Chromium effect respectively in mycorrhizal and non-mycorrhizalwheat plants. The dosages of metal were framed on the basis of recommended levels for plants. The application of *Glomus hoi* showed significant reduction in metal stress and better growth, biomass, improved physiological state and higher yields were recorded in mycorrhizal plants as compared to non-mycorrhizal plants. In general, AM fungi protected the wheat plants against metal toxicity and also improved the nutrients contents of plants. Thus the studyadvocates the use of AMFto mycoremediate metal toxicity and to have better crop yield.

Keywords:AM fungi, wheat, metal stress, mycoremediation.

Introduction

Ecosystem has been contaminated with heavy metals due to various natural anthropogenic activities. Metals may go into soil, water and human body via air, water, food or by skin absorption when they come in get in touch with human during industrial manufacturing process and agriculture practices. ArbuscularMycorrhizal Fungi (AMF) makes a symbiotic relationship with root of higher plants and provides a direct link between soil and plant roots. The AM fungi are the important rhizospheric microorganism, increase root and shoot biomass and improve plant growth. In somewhat metal contaminated areas, these AM fungi enhanced uptake of metals via shoot, while in rigorously contaminated soil, AM fungi lessen metals concentration in shoot and protects plant against detrimental effects of metals[1]. AM fungi not only contribute to plant growth mostly in disturbed and heavy metals contaminated sites by increasing access of the plants relatively to immobile nutrient minerals such as phosphorus but also fights against pathogens and predators[2].

These mycorrhizalfungi improve the physical and chemical properties of soil and helps in binding heavy metals to the plant roots that in turnobstruct their translocation into shoot.

Man induced chemical based technologies for soil remediation make the land futile and

restrains the plant growth as they confiscate all the essential biological activities. Therefore sustainable on-sites techniques need to be developed for the remediation of heavy metals. Mycoremediation is the use of fungi to stabilize, clean-up, low-cost environment friendly and potentially effective technology for the reclamation of polluted soils. Stratagem used by AM fungi consistof metalimmobilization by precipitating polyphosphate in the soil, fungal secretions, fungal cell wall adsorption andchelation of metals inside the fungus. Heavy metals cannot be degraded chemically and need to be physically immobilized or removed[3]. Wheat (*Triticumaestivum* L.) belonging to family-poaceae is one of the majorly grown cereal crop cultivatedon more land area than any other food crop throughout world.Wheat, eaten as the whole grainis an important source of carbohydrates, multiple nutrients and dietary fibre.

Association of AMF with crop plants to bioremediate toxic metals is an appealingnoteworthy area of research. Little information is available on changes in growth parameters of crop plants like wheat under metal stress and AMF applications. Engineering industries release various metals in their effluents which are finally discharged in environment. When this industrial effluent is discharged in nature then it causes pollution and long term toxic effects on biological life. Thus there is an urgent need of eco-friendly and cost effective remedial strategy. An initial survey and pilot project was done and effluent was collected from a specific engineering industry at Jaipur. Physico-chemical analysis of effluents was done and it was found that these effluents have high Fe (Iron) and Cr (Chromium) concentration. Soil was collected from the site where effluent was discharged and from it AMF were isolated and characterized. AMF biodiversity showed presence of *Glomus* species in high percentage, indicating its tolerance towards metal presence. This became the basis for selecting *Glomus* for present study. Keeping in view thisand the significance of AMF in mycoremediation of metals, this research study was plannedwith the following objectives:

- Culturing and inoculum preparation, determination of % root colonization and spore densities of AM fungi.
- Estimation of morphological and biochemical plant growth parameters in AM fungi inoculated and un-inoculated wheat plants
- Determination of Metal (Fe and Cr) uptake by AM fungi inoculated and un-inoculated Wheat Plant.

Material and methods

Mass culture of AMF

AM fungi species *Glomus hoi,* an obligate symbiont was obtained from TERI, New Delhi andmass multiplied on onion and wheat itself in plastic trays and pots containing soil and sand(3:1). After 45 days plants were uprooted and roots were boiled in 10% KOH than stained with Trypan blue inlactoglycerol for examining spores and fungal hyphae under microscope.

Spore count and density evaluation

Density evaluation of spore/10g of soil was done. For this 10 g of soil was dissolved in 100ml of water which was kept still for 10-15 minutes till water movement seizes. Then it was sieved through mesh sieves. Spore counting was done using wet sieving and decanting technique[4] by Gerdemann and Nicolson(1963). The root pieces retained in 710um were examined under dissecting microscope for attached hyphae, spores and

sporocarp. The organic matter from 250um sieve was examined for sporocarp and large spores. The 105um yielded most spores smaller than 100um, often occurring in clusters and mostly smaller detached spores. The spore density was 150-200 /10g of soil used in the following experiments.

Plant material

Wheat seed variety Raj4238 was obtained from the Rajasthan Agricultural Research Station, Durgapura, Jaipur. All Seeds were surface sterilized (10 min, 1% Hgcl2) and tenderly washed five times by deionized water and germinated in small plastic pots for a week. These were selected for uniformity before sowing. Young seedlings were transplanted in pots and the plants were allowed to grow for 80 days.

Fungal inoculums

tThe AM fungi used was *Glomushoi* with dry soil substrates obtained from the AMF culture maintained. AMF spores in dry sand-soil mixture were used in mycorrhizal inoculated plants. Each pot was supplied with 50 g of AM fungal inoculum having approximately 1000 spores per pot. AMF inoculum was provided during the seedling transplantation process and was not given in nonmycorrhizal plants.

Pot experiment and growth conditions

This experiment was conceived in pot trials under natural conditions to determine the potential of AM fungi in mycoremediation of heavy metals- Iron and chromium. There were two sets of experiment– set Iand set II were designed to study effect of Iron and chromium respectively in mycorrhizal and non-mycorrhizal wheat plants. For this $FeSo_4$and $K_2Cr_2O_7$ have been used. The dosages of metals were selected on the basis of recommended levels of metal for plants.The experiment was conducted in completely randomized design using wheat variety Raj4238. The experiment was replicated three times. Each pot contained 5kg soil along with 50g of AMF inoculum in mycorrhizal treatments, while the same amounts of soil was added to non-mycorrhizal treatments without AMF inoculum. The treatments were either inoculation or non-inoculation of the AM fungi and the addition of three iron concentrations to the soil (100, 300 and 600mg/kg) and three chromium concentrations to the soil (100, 200 and 300mg/kg) respectively. Plants were harvested after 80 days for analysis. Roots and shoots of the harvested wheat plants were rinsed with tap water to remove soil particles and then carefully washed with deionized water for further study. The two experimental sets are given below.

Set I treatment combinations for the experiment are as follows;

T1: 100mg kg^{-1} soil (Fe at recommended levels)

T2: 300mg kg^{-1} soil (Fe at recommended levels)

T3: 600mg kg^{-1} soil (Fe at recommended levels)

T4: 100mg kg^{-1} soil (Fe at recommended levels) + Mycorrhiza (*Glomus hoi*)

T5: 300mg kg^{-1} soil (Fe at recommended levels) + Mycorrhiza (*Glomus hoi*)

T6: 600mg kg^{-1} soil (Fe at recommended levels) + Mycorrhiza (*Glomus hoi*)

T7: Un-inoculated control (No treatment)

Set II treatment combinations for the experiment are as follows;

T1: 100mg kg^{-1}soil (Cr at recommended levels)

T2: 200mg kg^{-1}soil (Cr at recommended levels)

T3: 300mg kg^{-1} soil (Cr at recommended levels)

T4: 100mg kg^{-1} soil (Cr at recommended levels) + Mycorrhiza (*Glomus hoi*)

T5: 200mg kg^{-1} soil (Cr at recommended levels) + Mycorrhiza (*Glomus hoi*)
T6: 300mg kg^{-1} soil (Cr at recommended levels) + Mycorrhiza (*Glomus hoi*)
T7: Un-inoculated control (No treatment)
A basal dose of Hoagland's Nutrient solution was applied to all treatment once per week. Normal recommended cultural practices were strictly followed for optimum plant growth. Plant measurement and analysis was done by methodsgiven below:

AM Root colonization

In Roots, AMF colonization was estimated after washing, clearing and staining[5]byusing the modified grid-line intersect method[6]. The stained roots were then mounted on glass and colonization percentage of mycorrhiza was estimated for each by examining 2 cm long, 100 root pieces.

Plant biomass

At the time of harvest, shoots and roots were separated. Samples of fresh shoot and root were taken to assess mycorrhizal colonization. Fresh weights of shoot and root were measured after rinsing with tap water and then with deionized water. Plants were weighed after oven drying at 60°C for 72 hours to estimate total shoot and root dry weight.

Biochemical Analysis

The chlorophyll pigments and carotenoids in the leaves were estimated following the method of Arnon[7] (1949). The fully expanded leaves from wheat were collected in the polythene bags. The leaves were washed out thoroughly with distilled water. Weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The acetone extract volume was made up to a known and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. Protein content in plant leaves (50g) was measured using Lowry*et al.,* method (1951)[8]. Sugar content in plant leaves (50g) was measured using Anthrone method[9].

Metal uptake

After dry weight determination, the oven dried tissue samples (shoots and roots) were grinded, acid digested and the metal contents (Fe and Cr) in plant tissues (shoot and roots) were determined by using atomic absorption spectrophotometer.

Statistical analysis

Data on morphological parameters, biochemical contents, metal concentration and AMF root colonization were analyzed with analysis of variance (ANOVA) technique using SPSS software. For significant F value, LSD test was used for mean comparison at 5% level.

Results and Discussion

Mycorrhizal colonization

Table 1 and4 shows the influence of Iron and chromium toxicity on Mycorrhizal (M) and Non-Mycorrhizal (NM) wheat shoot and root biomass (individual and total length, fresh and dry weight). Wheat plants were significantly affected by AMF inoculation with notably higher shoots and root biomass production. The biomass (both root and shoot) of Mplantswas higher than those of NMplantsfor each concentration of iron and chromium. In NM plants, the reduced biomass was observed with the increase of iron and chromium concentration. The same trend was observed in both shoot and root parts of plants. In 100 mgkg-1 soil, a significant increase ($P<0.05$) was noted in shoot and root biomass of M plants with *Glomushoi.* While the decrease in the trend was observed at 300 and 600 mg kg-1 concentration in both inoculated and non-inoculated plant shoots and roots.

Table 2 and 5 shows the percentage of AM fungi colonization with roots of wheat plants. The result indicated that AMF

colonization was not detected in non-inoculated treatments while all the inoculated treatments (M) showed high colonization rates with abundantly formed arbuscules, vesicles and hyphal structures. From the results, it is obvious that the symbiotic relationship between wheatand AMF can be well established under metal stress conditions. The high percentage of AMF colonization viz. 81.66 %, 78.66% and 73.33% appeared at the 100, 300 and 600mg/kg Fe concentration, while the low colonization 69.33 % appeared at 300 mg kg^{-1}Cr concentration. Mycorrhizal colonization of inoculated roots ranged between 60-70%. Metal addition negatively influenced AMF root colonization and it was found to diminish linearly with the increase of metal concentration in soil.

Effect on plant growth

Table1-6 shows the effect of increasing metal concentration that is iron and chromium on overall growth of M and NM wheat plants. M wheat plants exhibited better growth than NM wheat plants. Results showed that Fe concentration (300 and 600 mg kg^{-1}) caused reduction in plant growth parameters in both M and NM plants. At 100 mg kg^{-1} Fe, a significant improvement mainly in the M plants was observed. Generally, M plants exhibited significantly higher shoot and root length, fresh and dry weight than NM plants in non applied and 100mg kg^{-1}Fe. Meanwhile, non-inoculated plants exhibited slower growth with increasing Fe. The significant positive changes and better root and shoot growth were observed in M plants at Cr concentrations (0,100, 300 $mgkg^{-1}$) as compared NM plants. The reduction in plant root and shoot growth was observed at highest Cr concentration (300 mg kg^{-1}) in both M and NM plants.

Metal uptake in wheat plants

Table 2 and 5 shows the linear correlation in plant tissues between both metal concentration in soil and plant uptake that is increased Fe and Cr uptake with increasing soil metal concentration. M plants accumulated more concentration of metal in all Fe (100, 300, 600 mg kg^{-1}) and Cr (100, 200, 300 mg kg^{-1}) treatments than NM plants. The trend of metal concentrations in both M and NM plants was statistically different at all Fe and Cr concentrations except in control in which no metal was supplied. However, the increased concentration of Fe and Cr was observed in wheat NM plants as compared to M plants as the application dosage of both was increased during treatments. The more accumulation was observed at the highest Fe and Cr dosage application.

Plant biochemical Analyses

Table 2 and 5 shows the effect of mycorrhiza on relative chlorophyll and carotene contents with increasing metal concentrations. The concentration of chlorophyll and carotene contents in M plants was significantly higher than those of NM plants at each Fe concentration (100, 300, 600 mg kg^{-1}). The highest chlorophyll a and b contents were observed at Fe concentration of 100 mg kg^{-1}in both M and NM plants. The lowest chlorophyll and carotene contents were observed at Cr concentration (200 and 300 mg kg^{-1}) in both inoculated and inoculated treatments. The significant differences were found due to AMF inoculation in plants. M plants showed lower proline levels in control and 100 mg kg^{-1} Fe and Cr concentration.

Discussion

The study showed that AMF formed mycorrhizal symbiosis well in wheat plants under Fe and Cr stress. Some morphological and physiological parameters of wheat plants such as length, weight, chlorophyll,

carotene and proline could be improved by AMF species under metal toxicity. In this study, Fe and Cr stress had a strong effect on AMF development and colonization decreased with the increase of metal concentrations. Mycorrhizal colonization decreased as the concentration of metal increased in soil.Researchers have reported before that spores of *Glomus* species differed markedly in their sensitivities to Cd, Pb, and Zn exposures. *G. mosseae* was reported to be more sensitive at diverse levels of metal in the soil[10]. Contradictory to this, it was also reported that Cd toxicity does not have an effect on the root colonization by AM fungi. These observations demonstrate that different levels of compatibility between host plants and AMF isolates may occur under diverse conditions of metal toxicity in soil[11].

The toxic effects of Fe and Cr have widely been studied in different plant species and both are known to reduce or inhibit plant growth. In the present work, with increasing Fe and Cr in the soil, shoot and root length as well as shoot and root fresh and dry weights were decreased in NM plants. The same results were reported prior also. However, AMF associations cause to increase the shoot, root length and weight, chlorophyll and carotenoides concentration. These positive effects were associated to the mycorrhiza contribution in uptake of host mineral nutrient especially immobile soil nutrients and the resistance of metal uptake[13].

Mycorrhizas enhance the metal tolerance of host plants in soils containing high concentrations of toxic metals. In the present study, Fe and Cr concentrations in the shoots and roots of wheat plants were always decreased by mycorrhizal inoculations to different degrees independent of the soil metal concentrations. A possible mechanism of this effect is the ability of AMF to bind heavy metals by fungal hyphae outside and inside the roots. Numerous experimental studiespreviously done have indicated that in heavy metal contaminated soils, mycorrhizal plants usually had higher root metal concentrations but lower shoot metal concentrations compared with nonmycorrhizal plants[14]

Inoculation of plant species including soybeans, maize and lettuce with AM fungi decreases Zn and Cd concentrations in plant leaves at high soil metal concentrations and increases metal concentrations of plant leaves at low soil metal concentrations. Metal concentration in roots was more than that of soil metal, indicating that the metal absorption mechanism for roots is an active process in wheat. It is suggested that the mechanisms of metal absorption in roots and xylem loading are related to an energy dependent active process[15]. This elevated metal accumulation was seen in mycorrhizae exposed to high levels of Zn. This might be due to the reason that different AMF ecotypes can exhibit different degrees of metal tolerance. It can be attributed to fact that isolates from habitats contaminated with heavy metals are generally more metal tolerant than isolates from non-contaminated soils. The result of the study indicated that AMF inoculation enhanced the chlorophyll formation in wheat plants. The alterations in chlorophyll content can be due to the result of nutrient deficiencies and the reaction of plants to the environments in which they survive. This chlorophyll deficiency might be due to metal induced oxidative stress[16].

It was also reported the chlorophyll content of non mycorrhizal wheat plants is reduced under Cd stress which may affect the synthesis of chlorophyll enzyme, thereby, reducing the photosynthesis of the plants and reduce the growth of plants. This might

be due to the fact that wheat plants especially mycorrhizal plants could increase the ability to withstand adversity by delaying protein degradation and maintaining normal metabolism of proteins[17]. These are in agreement to results obtained in the present study in which wheat with AMF showed better growth parameters and high metal accumulation. Arbuscularmycorrhizal fungi are proposed for improving yield because it is known that mycorrhizal roots acquire P more efficiently than non-mycorrhizal roots especially at low soil fertility levels[18]. Mycorrhizas enhance the metal tolerance of host plants in soils containing high concentrations of toxic metals[19].

The effect of AMF on the uptake of heavy metals by plants is not uniform, as both increases and decreases have been reported. The outcome probably depends on the selected plant and also on the species and strain of the fungus used. The majority of plants without AMF do not survive transplanting into contaminated soil. Those that survive grow badly, and accumulate higher concentrations of chromium, both in the shoots and also in the roots due to mycorrhizae. These results were in accord to other reports that the heavy metal was strongly retained in the root system[20]. The effect of AMF in decreasing heavy metal stress has been assigned to the selective immobilization of the toxic metal within the root tissues that are colonised by the fungus[21] or to the high metal sorption capacity of the extraradical mycelium of the AMF[22].

Plant biochemical analysis

Fig 4 a, b and c shows the effect of mycorrhiza on relative chlorophyll and carotene contents with increasing Fe and Cr concentrations. The concentration of chlorophyll (4a and b) and carotene contents (4c) in M plants were significantly higher than those of NM plants at each Fe concentration (100, 300, 600 mgkg- 1). The highest chlorophyll a and b contents were observed at Cr concentration of 100 mg kg-1 in both M and NM plants. The lowest chlorophyll and carotene contents were observed at Cr concentration (200 and 300 mgkg-1) in both inoculated and inoculated treatments. Similarly to our results, an alleviation effect of AMF in front of Zn toxicity, decreasing the Zn translocation towards the shoot was also earlier observed[23]. Researchers have suggested a direct effect of the AMF through adsorption and binding of the heavy metal in the mycorrhizosphere and an indirect effect through the improvement of the plant nutrition. AMF is found to be associated with not only cereal crops but other plants of economic importance growing in arid and semi arid zone also like date palms[24]. Thus AMF can be exploited, but mycorrhizal plants as a tool in phytoremediation strategies needs further research to understand the mechanisms involved in the plant's protection against metal toxicity. These research efforts will help to integrate this biotechnology in agricultural and environmental engineering processes.

Conclusion

It is concluded from the results of the present study that the symbiotic relationship between the wheat plants and AMF was well established under metal stress. AM colonization increased the biomass, growth of plants and availability of essential nutrients to wheat plants. Bioremediation is the use of biological organisms to remediate or clean up contaminated soils to remove or stabilize heavy metals. AM fungi are important in mycoremediationas they play a vital role in metal tolerance and accumulation. The study concluded that

inoculation of mycorrhizal fungal species in plants had beneficial impacts and better growth, biomass, improved nutrients uptake and higher yields were recorded in M treated plants as compared to NM treated plants. Broadly, AMF not only protected the wheat plants against metal's toxic effectsbut also improved the nutrient status of plants. The importance of mycorrhizafor wheat plants under high Fe and Cr concentrations was observed. As levels of heavy metal is increased due to increase use of sewage sludge in agriculture, and also due to industrial emissions that cause to contaminate the agricultural soils, there is a current need to remediate. The practical application of AMF association with cereal crops in the field can developsustainable agriculture and ecosystem. Furthermore, experiments under field conditions should be employed to study the extent to which mycorrhizal fungi can alleviate metal plant toxicity.

Acknowledgement

We thank the IIS (deemed to be University), Jaipur for financial assistance and providing necessary facilities.

T	Shoot length (cm)	Root length (cm)	Plant Height (cm)	Shoot fresh Weight (g)	Shoot dry Weight (g)	Root fresh Weight (g)	Root dry Weight (g)
T1	60.40±1.05*	20.90±0.46*	81.30±1.28*	15.95±0.09*	1.97±0.08*	4.97±0.10*	0.69±0.03*
T2	62.36±0.52*	22.70±0.66*	85.06±0.21*	16.42±0.18*	2.12±0.06*	5.33±0.33*	0.77±0.01*
T3	34.40±5.02*	15.20±1.40*	49.60±6.46*	11.10±0.15*	1.12±0.14*	1.67±0.36*	0.28±0.04*
T4	64.43±1.37*	25.16±1.50*	89.60±2.70*	17.04±0.56*	2.67±0.05*	5.35±0.14*	0.89±0.03*
T5	67.00±1.20*	25.50±1.04*	92.50±2.25*	17.33±0.11*	2.83±0.07*	5.84±0.05*	0.94±0.02*
T6	66.46±0.86*	26.76±0.92*	93.23±0.98*	17.87±0.16*	2.91±0.12	6.42±0.21*	0.95±0.03*
T7	71.90±2.80*	28.93±0.72*	100.83±2.1*	19.55±0.14*	2.84±0.21*	6.93±0.38*	1.02±0.03*

The data shown are the means ± standard error (n = 3). Value within each column marked with different * means values are * = significant and ** = not significant at $p<0.05$.Where, T: treatments, T: T1: 100mg kg^{-1} soil (Fe), T2: 300mg kg^{-1} soil (Fe), T3: 600mg kg^{-1} soil (Fe), T4: 100mg kg^{-1} soil (Fe) + (*Glomus hoi*), T5:300mg kg^{-1} soil (Fe)+ (*Glomus hoi*), T6:600mg kg^{-1} soil(Fe)+ (*Glomus hoi*), T7: Un-inoculated control (No treatment).

Table 1: Effects of increasing Iron concentration on morphologicalparameters of mycorrhizal and non-mycorrhizal wheat variety Raj4238.

T	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	TotalChlorophyll (mg/g)	Carotenoids (mg/g)	Metal concentration (mg/kg)	AMF colonization (%)
T1	13.07±1.66*	5.05±0.71*	16.85±1.87*	0.61±0.07**	0.46±0.01*	0.00±0.0**
T2	12.42±1.68*	4.09±0.95*	16.65±2.29*	0.46±0.02**	0.70±0.01*	0.00±0.0**
T3	7.82±0.99*	2.65±0.12**	10.47±1.08*	0.37±0.16**	0.90±0.01*	0.00±0.0**
T4	14.84±0.71*	5.50±1.90*	20.34±1.85*	0.59±0.05**	2.60±0.02*	78.66±0.88*
T5	14.52±2.23*	5.70±0.69*	20.23±2.90*	0.76±0.17**	7.09±0.02*	81.66±1.76*
T6	12.66±1.78*	7.17±1.20*	19.83±2.86*	0.53±0.05**	5.04±0.01*	73.33±4.05*
T7	15.84±0.28*	10.50±0.67	26.15±0.63*	0.57±0.08**	0.00±0.00*	0.00±0.0**

The data shown are the means ± standard error (n = 3). Value within each column marked with different * means values are * = significant and ** = not significant at $p<0.05$. Where, T: treatments,T1: 100mg kg^{-1} soil (Fe), T2: 300mg kg^{-1} soil (Fe), T3: 600mg kg^{-1} soil (Fe), T4: 100mg kg^{-1} soil (Fe) + (*Glomus hoi*), T5:300mg kg^{-1} soil (Fe)+ (*Glomus hoi*), T6:600mg kg^{-1} soil(Fe)+ (*Glomus hoi*), T7: Un-inoculated control (No treatment).

Table 2: Effects of increasing Iron concentration on biochemical parametrs of mycorrhizal and non-mycorrhizal wheat variety Raj 4238.

Treatments	T1	T2	T3	T4	T5	T6	T7
T1	-	NS	Sb	Sa	Sa	Sa	Sa
T2	NS	-	NS	Sa	Sa	Sa	Sa
T3	Sb	NS	-	Sa	Sa	Sa	Sa
T4	Sa	Sa	Sa	-	Sa	Sa	Sa
T5	Sa	Sa	Sa	Sa	-	Sc	Sa
T6	Sa	Sa	Sa	Sa	Sc	-	Sa
T7	Sa	Sa	Sa	Sa	Sa	Sa	-

Where, values are significant at Sa=0.001, Sb=0.01, Sc=0.05, Where, T1: 100mg kg^{-1} soil (Fe), T2: 300mg kg^{-1} soil (Fe), T3: 600mg kg^{-1} soil (Fe), T4: 100mg kg^{-1} soil (Fe) + (*Glomus hoi*), T5:300mg kg^{-1} soil (Fe)+ (*Glomus hoi*), T6:600mg kg^{-1}soil(Fe)+ (*Glomus hoi*), T7: Un-inoculated control (No treatment).

Table 3: Multiple comparisons (Post-Hoc-LSD) for plant growth parameters under Metal (Fe) application.

T	Shoot length (cm)	Root length (cm)	Plant height (cm)	Shoot fresh Weight (g)	Shoot dry Weight (g)	Root fresh Weight (g)	Root dry Weight (g)
T1	53.26±1.18*	24.66±1.01*	77.83±2.01*	16.00±1.55*	2.09±0.08*	4.29±0.87*	0.68±0.02*
T2	49.06±0.69*	24.13±1.95*	73.20±2.45*	14.70±0.37*	1.96±0.10*	3.72±0.32*	0.54±0.04*
T3	41.80±1.11*	18.63±1.87*	60.43±2.73*	11.33±0.75*	1.44±0.24*	2.13±0.11*	0.26±0.02*
T4	61.93±0.76*	28.03±1.72*	89.96±1.97*	20.80±0.68*	2.90±0.23*	6.83±0.17*	0.92±0.02*
T5	60.60±0.89*	27.46±1.44*	88.06±1.88*	19.55±0.59*	2.65±0.23*	6.80±0.11*	0.84±0.06*
T6	58.53±1.22*	25.40±0.94*	83.93±1.75*	18.13±0.68*	2.42±0.19*	6.23±0.33*	0.84±0.03*
T7	64.10±0.60*	32.13±1.60*	96.23±2.19*	21.66±0.46*	3.27±0.32*	6.92±0.20*	0.95±0.03*

The data shown are the means ± standard error (n = 3). Value within each column marked with different * means values are * = significant and ** = not significant at $p<0.05$.Where, T: treatments,T1: 100mg kg^{-1} soil(Cr), T2: 200mg kg^{-1} soil (Cr), T3: 300mg kg^{-1} soil (Cr), T4: 100mg kg^{-1} soil (Cr) + *Glomus hoi*, T5: 200mg kg^{-1} soil (Cr) + *Glomus hoi*, T6: 300mg kg^{-1} soil (Cr) + *Glomus hoi*, T7: Un-inoculated control (No treatment).

Table 4: Effects of increasing chromium concentration on morphologicalparameters of mycorrhizal and non-mycorrhizal wheat variety Raj 4238.

T	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Carotenoids (mg/g)	Metal (Cr) Concentration (mg/kg)	AMF Colonization (%)
T1	12.00±1.80*	8.17±1.22*	20.17±2.78*	0.71±0.07**	0.32±0.11*	0.00±0.0**
T2	12.98±1.73*	8.16±1.87*	21.34±3.35*	0.67±0.08**	0.38±0.34*	0.00±0.0**
T3	12.07±1.66*	6.05±0.71*	18.12±2.35*	0.63±0.05**	0.46±0.01*	0.00±0.0**
T4	12.51±3.05*	6.50±1.90*	23.68±2.17*	0.69±0.05**	1.23±0.17*	74.66±2.84*
T5	11.84±0.13*	5.09±0.95*	20.02±1.88*	0.56±0.02**	2.15±0.02*	73.33±3.28*
T6	14.86±1.95*	6.37±0.51*	23.90±0.76*	0.61±1.69**	2.25±0.05*	69.33±2.90*
T7	17.82±0.99*	13.32±0.59*	31.14±1.32*	1.13±0.49**	0.00±0.00*	0.00±0.0**

The data shown are the means ± standard error (n = 3). Value within each column marked with different * means values are * = significant and ** = not significant at $p<0.05$.Where, T: treatments,T1: 100mg kg^{-1} soil(Cr), T2: 200mg kg^{-1} soil (Cr), T3: 300mg kg^{-1} soil (Cr), T4: 100mg kg^{-1} soil (Cr) + *Glomus hoi*, T5: 200mg kg^{-1} soil (Cr) + *Glomus hoi*, T6: 300mg kg^{-1} soil (Cr) + *Glomus hoi*, T7: Un-inoculated control (No treatment).

Table 5: Effects of increasing chromium concentration on biochemical parameters of mycorrhizal and non-mycorrhizal wheat variety Raj 4238.

Treatments	T1	T2	T3	T4	T5	T6	T7
T1	-	Sa	Sa	Sa	Sa	Sa	Sa
T2	Sa	-	Sa	Sa	Sa	Sa	Sa
T3	Sa	Sa	-	Sa	Sa	Sa	Sa
T4	Sa	Sa	Sa	-	Sa	Sa	Sa
T5	Sa	Sa	Sa	Sa	-	Sa	Sa
T6	Sa	Sa	Sa	Sa	Sa	-	Sa
T7	Sa	Sa	Sa	Sa	Sa	Sa	-

Where, values are significant at Sa=0.001, Sb=0.01, Sc=0.05The data shown are the means ± standard error (n = 3). Value within each column marked with different * means values are * = significant and ** = not significant at $p<0.05$.Where, T1: 100mg kg^{-1} soil(Cr), T2: 200mg kg^{-1} soil (Cr), T3: 300mg kg^{-1} soil (Cr), T4: 100mg kg^{-1} soil (Cr) + *Glomus hoi*, T5: 200mg kg^{-1} soil (Cr) + *Glomus hoi*, T6: 300mg kg^{-1} soil (Cr) + *Glomus hoi*, T7: Un-inoculated control (No treatment).

Table 6: Multiple comparisons (Post-Hoc-LSD) for plant growth parameters under Metal (Cr) application.

References

1. Khan AG, Kuek C, Chaudhry TM, Khoo CS and Hayes WJ 2000, Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere.***41** 197-207.
2. Sharma A 2013, Comparative efficacy of nematicides with VAM fungi against *Heteroderaavenae* infecting wheat. *Intern. J. of Biotech. Res.***3**(1) 11-16.
3. Kedia R and Sharma A 2015, Bioremediation of industrial effluents using arbuscularmycorrhizal fungi. *Biosci. Biotech. Res. Asia.***12**(1) 197-200.
4. Gerdemann JW and Nicolson TH 1963, Spores of mycorrhizal*Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.***46**235-244.
5. Koske RE and Gemma JN 1989, A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research.***92** 486-488.
6. Giovannetti M and Mosse B 1980, An evaluation of techniques for measuring Vescicular-ArbuscularMycorrhizal infection in roots. *New phytologist.***84** 489-500.
7. Arnon DI 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *beta vulgaris*. *Plant physiology.***24** 1-15.
8. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ 1951, Protein measurement with the Folin phenol reagent. *J. of Biological Chemistry*. **193**265-275.
9. Yemm EW and Willis AJ 1954, The estimation of carbohydrates in plant extracts by anthrone. *The Biochemical journal.* **57**(3) 508–514.
10. Pawlowska TE and Charvat I2004, Heavy-metal stress and developmental patterns of arbuscularmycorrhizal fungi. *Appl.Env. Microbio.***70** 6643-6649.
11. Shahabivand S, Zaremaivan H, Mohammadi GE, Sharifi M and Aliloo AA2012, The effects of root endophyte and arbuscularmycorrhizal fungi on growth and cadmium accumulation in wheat under cadmium toxicity. *Plant Physiol. and Biochem.***60** 53-58
12. Kanwal S, Bano A and Malik RN 2015, Effects OfArbuscularMycorrhizal Fungi on Wheat Growth, Physiology, Nutrition And Cadmium Uptake Under Increasing Cadmium Stress. *Int. J. Agri. and Agri. R.***7**(5) 30-42.

13. Hu Y, Rillig MC, Xiang D, Hao Z and, Chen B2013, Changes of AM fungal abundance along environmental gradients in the arid and semi-arid grasslands of northern China. *Plos one.***8**(2) 1-10.
14. Chen X, Chunhua W, Jianjun T and Shuijin H2005,Arbuscularmycorrhizae enhance metal lead uptake and growth of host plants under a sand culture experiment. *Chemosphere.***60**665-671.
15. Ueno K, Nomura S, Muranaka S, Mizutani M, Takikawa H and Sugimoto Y2011,*Ent*-2′-*epi*orobanchol and its acetate, as germination stimulants for *Strigagesnerioides*seeds isolated from cowpea and red clover**.** *J. of Agri.and Food Chem***.59** 10485-10490.
16. Gallego SM, Benavides MP and Tomaro M1996, Effect of heavy metal ion excess on sunflowers leaves: evidence for involvement of oxidative stress. *J. of Plant Sci***.121**151-159.
17. Wu QS and Xia RX2006,Arbuscularmycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *J. of Plant Physiol.***163** 417-425.
18. Sharma A and Trivedi PC 2019, Effect of arbuscularmycorrhizal fungi on life-cycle of *Heteroderaavenae* infecting wheat.*Asian J.ofMicrobio. Biotech. andEnv. Sci.***21**(5) 1010-1014.
19. Gohre V andPaszkowski U2006, Contribution of the arbuscularmycorrhizal symbiosis to heavy metal phytoremediation. *Planta journal.***223**8.
20. Gaur A andAdholeya A2004, Prospects of arbuscularmycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Int.J. of Cur. Sci***.86** 528–534.
21. Kaldorf M, Kuhn AJ, Schröder WH, Hildebrandt U and Bothe H1999, Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscularmycorrhizalfungus. *J. Plant Physiol***.154** 718-728.
22. Joner EJ, Briones R andLeyval C 2000, Metal binding capacity of arbuscular mycorrhizalmycelium. *Plant Soil.***226** 227-234.
23. Christie P, Li X and Chen B 2004,Arbuscularmycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc. *Plant Soil.***262** 209-217.
24. Sharma A and Yadav S 2014, Review on role of VAM fungi in crop plant-soil system.*Int. J. of Agri. Sci. and Res.* **3**(1) 17-23.