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BIOREMEDIATION OF HEAVY METALS FROM ENGINEERING INDUSTRY EFFLUENTS USING AM FUNGI AND ORGANIC AMENDMENTS

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ABSTRACT

The present study was under taken to examine the effects of Arbuscular Mycorrhizal fungi (AMF) *Glomus hoi* and other available potential organic amendments on heavy metal (HM) found in abundance in engineering industry effluent on growth parameters of host wheat (*Triticum aestivum* L.) variety Raj4238 underpot trials. Two sets of experiments were planned to study effect of Iron and Chromium effect respectively in mycorrhizal and various organic amendments treated wheat plants. The dosage of both metals was framed on the basis of recommended levels for plants. The application of *Glomus hoi* showed significant bioremediation of metals and better growth, biomass, improved physiological state, followed by Neem oil cake, Rhizobacteria and Farm yard manure (FYM). In general, AM fungi accumulated high metal content in the host plants, protected them against metal toxicity and also improved the nutrients contents of plants. Thus, the study campaigns the use of AMF as compared to other organic amendments to bioremediate metals from sites where industrial effluents are discharged and to have better and safe soil, water environment.

KEY WORDS: AM fungi, Organic amendments, Wheat, Heavy Metals, Bioremediation.

INTRODUCTION

The brisk industrial growth over the last three decades has enormously contributed to increase in waste effluents and their untreated getaway in environment, which in turn has caused elevation in pollution and ecosystem imbalance. It has also resulted in augmented stress on natural resources and has become a number one source of immeasurable contamination in the environment. One of the major tribulations around the world right now is environment pollution and its remediation. Wide-ranging pollutants viz. industrial effluents, heavy metals, hydrocarbons, pesticides, dyes etc. are the main players, which are chiefly accountable for polluting environment and creating a task/ challenge of eliminating residual contaminants.

Biological clean-up methods have a diversity of applications such as cleansing of contaminated areas such as soil, water, slush/sludge and flow from different sources. Quite a few methods were intended and created, but mostly either the procedure reproduced derivative contamination in environment or it wasn't so effective. Bioremediation is a process which utilizes the use of living organisms, like microbes and bacteria to treat and remove pollutants, toxins and contaminants from water, soil and subsurface, by not only varying the environmental conditions but by also stimulating microbial growth and degrading the intended pollutants from. Bioremediation, an efficient, environmentally friendly and striking means to treat and reprocess our environment is used throughout the globe, with varying degree of achievement. Applying the right microbial treatment to a site for bioremediation would definitely offers a lucrativetechnique and bid significant progress prospects in this area. Bioremediation has a potential to recuperate the contaminated sites back to their actual form. So far only a petite amount of culturable microorganisms have been used and still a diverse range of microbial communities are left to be explored. Arbuscular mycorrhizae, primarily concerned in phytoremediation are root colonizing symbiotic fungi that utilize plants for soil remediation. Also, there is crucial role of AMF in soil amendment especially in moderation of heavy metals and AMF express pure soil and plants roots and shoots associations and also encourage nutrient and biomass augmentation (Sharma *et al.,* 2014). With the progress done in this field of research, mechanisms used byAMF to protect host and bioremediate heavy metal are- heavy metal ions immobilization and retention within AMF structures; to prevent metal uptake occurrence of chelation, precipitation, and complex formation in rhizosphere; for the cytosolic storage, usage of organic acids, metallothione and compound-specific chaperones; heavy metals sequestration within AMF arbuscules and plant vacuole; enhanced activation of antioxidation system; using diverse transporters or pore in plasma membrane, active and passive transport of the heavy metal; further using specific and nonspecific mechanisms, heavy metal ions dissemination occurs (Mishra *et al.*, 2019).

MATERIALS AND METHODS

The Engineering industry effluent was collected from National Engineering Industries (NEI) located Khatipura Road, Shanti Nagar, Ganpati Nagar, Jaipur, Rajasthan in clean 10 L plastic bottles and maintained at 4 degree temperature. The effluent was collected from outlet pipes of factories at the effluent collecting point after primary treatment. The samples were collected three times and composite sample was prepared. The collected effluent was analysed for various physical and chemical parameters in SCS Enviro Servics, Jagatpura, Jaipur, Rajasthan. Soil samples were collected from four different sites where effluent was discharged. To characterize and study, AM fungal spores of the collected soil samples were isolated using wet sieving and decanting technique by Gerdemann and Nicolson (1963). The true seeds of wheat cultivar variety Raj-4238 were procured from the RARI, Jaipur for experimental work. 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. The entire experiment was arranged under pot trials in a randomized block design with three replicatesto determine the potential of AM fungi in mycoremediation of heavy metals- Iron and chromium which were found in high concentration in engineering industry effluent. There were two sets of experiment– I and II which were designed to study effect of Iron and chromium respectively in mycorrhizal and non-mycorrhizal wheat plants along with organic amendments. For this $FeSo_{4.}7H_20$ and $K_2Cr_2O_7$ have been used. The dosages of metals were selected on the basis of recommended levels. 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. Three types of natural fertilizers viz. neem oil cake and farm yard manure (FYM) along with AMF and high metal dose were applied to study metal bioremediation and tolerance of wheat.

Set I treatment combinations for the experiment are as follows:

T1: 600mg kg-1 soil (Fe at recommended levels)

T2: 600mg kg-1 soil (Fe at recommended levels) + FYM@6t/h (Farm yard manure)

T3: 600mg kg-1 soil (Fe at recommended levels) + Neem oil cake@4t/h

T4: 600mg kg-1 soil (Fe at recommended levels) + Mycorrhiza (*Glomus hoi*)

T5: 600mg kg-1 soil (Fe at recommended levels) + Rhizobacteria

T6: Un-inoculated control (No treatment)

Set II treatment combinations for the experiment are as follows:

T1: 300mg kg-1 soil (Cr at recommended levels)

T2: 300mg kg-1 soil (Cr at recommended levels) + + FYM@6t/h (Farm yard manure)

T3: 300mg kg-1 soil (Cr at recommended levels) + Neem oil cake@4t/h

T4: 300mg kg-1 soil (Cr at recommended levels) + Mycorrhiza (*Glomus hoi*)

T5: 300mg kg-1 soil (Cr at recommended levels) + Rhizobacteria

T6: Un-inoculated control (No treatment)

A basal dose of Hoagland's Nutrient solution was applied to all treatment once per week. Standard suggested cultural methods were firmly practised for optimal host development. Host parameter's measurement and analysis was carried with suitable methods. The effect of AM fungi, metal stress and combinational (AM and metal stress) were analyzed for various morphological, biochemical and enzymological parameters for each treatment. 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.

Morphological, biochemical and physiological parameters

The plant shoot, root and total length of plants from each treatment was recorded in cm, the dry and fresh and weight of hostwere noted. 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.

The methodology used for physiological, biochemical and enzymological parameteranalyses is concisely explained underneath.The chlorophyll pigments and carotenoids in the leaves were estimated following the method of Arnon (1949). The Soluble Sugar was estimated by Anthrone method (Yemm and Willis, 1954). Using suitable methods estimation of Proteins (Lowry *et al.,* 1951), Proline (Bates *et al.,* 1973), Superoxide dismutase SOD (Dhindsa *et al.,* 1981), POD (Chakraborty *et al.,* 1993) and CAT activity (Chance and Machly, 1955) was carried.

Estimation of AMF Root colonization, Spore count and density

For host plant wheat, root and soil were mixed together in sample, packed and labelled in polyethylene bags for analysis. The soil samples were air-dried at room temperature. Washing of roots was done to remove soil particles, then preserved with FAA. For root colonization measurement, they were cleared in 10% KOH and placed in a water bath (90 °C) for up to 30 min. In Roots, AMF colonization was estimated after washing, clearing and staining (Koskeand Gemma, 1989) by using the modified grid-line intersect method and fixed by FAA solutions. 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. Root samples were prepared with 10% KOH and stained with 0.05% trypan blue lactophenol (Phillips and Hayman, 1970), and observed for root colonization (Giovannetti and Mosse,1980) under a microscopefor the presence of AM fungi.

Rhizospheric soil form wheat was collected with care, not loose and injure AMF spore and was evaluated. Density evaluation of spore/50g of soil was done. For this 50 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.

Metal uptake by plant material

Metal content in the various parts of the plants was estimated with the help of atomic absorption spectrophotometer (AAS). 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. The plant materials were oven dried to a constant dry weight and digested in a ternary acid mixture of nitric acid: perchloric acid: sulphuric acid (10:4:1). One gram of the plant material was digested at a temperature of 180 °C for 15 min. After the digestion was complete the volume was made up to 100 ml with distilled water and the AAS (Perkin Elmer model 1100) reading was noted.

Statistical analysis

The results obtained in the experiments were expressed in terms of mean value and Standard deviation. One-way ANOVA was used for data analysis. Results in each experiment were construed based on probability (p-value) and less than 0.05 and 0.01 was considered significant and highly significant, respectively.

RESULTS AND DISCUSSION

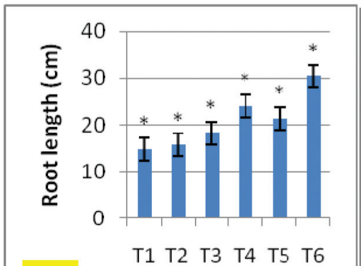
The engineering industryeffluent samples were subjected to characterization by performing various physical and chemical methods. The effluent samples were found to be high in parameter values such as BOD, COD, oil and grease, Fe, Cr and Ni. The species found in abundance was *Glomus,* then were*Acaulospora*and *Diversispora.*The *Glomus* species was found to be dominant than other observed AMF species.Thus, *Glomus hoi* which was procured from TERI, New Delhi, India was used for further studies. The results, it is obvious that the symbiotic relationship between *Triticum aestivum* and AMF can be well established under metal stress conditions. In set III and IV the optimum percentage of AMF colonization viz. 79.33 % and 78.66% appeared at the 600 mg kg^{-1} (Fe at recommended levels) and 300mg kg^{-1} soil (Cr at recommended levels) concentration. Not much difference was observed in spore density which was around 253/50g of soil and 259/50g of soil for Fe and Cr treated mycorrhizal plants. The data reported in the present work clearly indicated that the symbiotic relationship between wheat and AMF can be established under heavy metal stress conditions. The results presented that Fe and Cr are enthused AMF colonization percent in the little heavy metal concentration and shortened its concentration. These results are in treaty with Dhalaria *et al.*,(2020) who found mycorrhizal colonization or infection rate had been shown to be delayed, reduced, and even eliminated by high concentrations of metal.

In set Iand II, host *Triticum aestivum* var. Raj 4238 plants were treated with diverse group of organic amendments to bio-remediate metals- iron and chromium and to study differences in their potential for the same. The dissimilarity induced by different organic amendments as compared to AMF was studied for morphological parameters as mentioned above. Over all it was observed, that for all the amendments which were used here, plant height, shoot and root length, their fresh and dry weight also increases significantly with mycorrhizal, FY manure, Oil seed cake and rhizobial applications in metal treated plants. Although, the extent to which improvement laid by these organic amendments in host was quite variable. Thus, over all one can say that Metal has adverse effect on all morphological parameters of host and when organic amendments were applied host parameters were improved and best results were given by AMF. The metal accumulated by these was evaluated and it was observed that AMF treated wheat has highest concentration of Fe and Cr. Herein both the treatment (Fe and Cr), the metal's toxic effect was reduced to different extent on plant's morpho-metrics. Past analyzing different parameters, it was concluded that, after AMF, Rhizobacterial application, then neem oil cake and then FYM showed improvement in wheat plant. AMF was most efficient, whereas there was distinct difference between neem oil cake and rhizobacteria but FYM was quite near to only metal treated plants. So FYM although enhanced growth parameters but not up to much extend. The forthcoming of *Pseudomonas* and neem oil cake for enhancing elimination of chromium from contaminated soil was toured also prior (Govarthanan *et al.*, 2019).

In set I and Set II, where AMF, FYM, Neem oil cake and Rhizobacteria were applied along with metals (Fe and Cr) separately to study their comparative effect in bio-remediating metals, it was observed that AMF showed superior results in enhancing chlorophyll a, b and total, followed by rhizobacteria, neem oil cake and FYM. The results of present study are supported by the previous reports on wheat and other plants. In mycorrhizal treated plants of potato, Shinde and Khanna (2014) testified upsurge in contents of chlorophyll a, chlorophyll-b and total chlorophyll as compared to control.

For both metals it was observed that best results were given by AMF (3.19mg kg^{-1} in T4 treatment) for Fe and AMF (2.17mg kg^{-1} in T4 treatment) for Cr in set I and II as shown in Figures 1-30. FY manure, Neem oil cake and Rhizobacterial application showed metal accumulation at par with each. It was also observed that although they have not accumulated much metal in plants but they have decreased metal concentration in plant as compared to plant with only metal dose without any amendment. So, one can conclude that these may have enhanced plant tolerance and reduced toxic effects of metals on plants.These results are in accord with related previous studies (Ghosh *et al.*, 2009). Several studies conducted during the last few years have shown that AMF, such as *Glomus mosseae* and *Rhizophagus irregularis* exhibited improved heavy metal translocation in the plant shoot (Ali *et al.*, 2015).

For both sets, there was an increase in proline content. The combination treatments with AMF, FY manure, Neem oil cake and Rhizobacterial application along with metal showed variations in sugars content. Li*et al.*, (2019) stated that the incidence of AMF amplified plant entire dry weight, root activity, and sugar concentration at soil water content in ryegrass. Similarly, for protein comparative efficacy of AMF was also testedfor Fe and Cr with different organic amendments. In set I (Fe treated) and II (Cr treated), after AMF (for Fe highest was 21.91 mg g^{-1}; for Cr highest was 17.44 mg g^{-1}), most effective were rhizobacterial applications, neem oil cake and then FYM. Although



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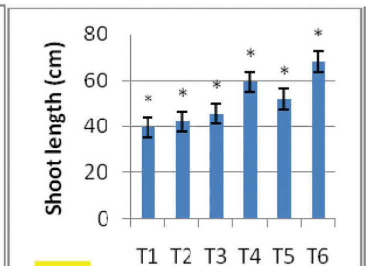

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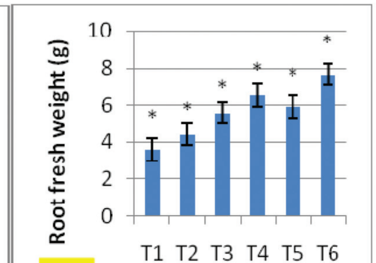

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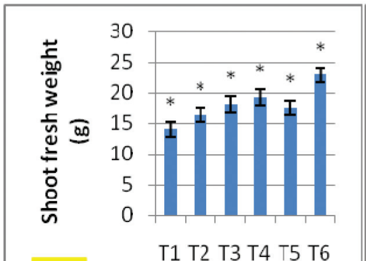

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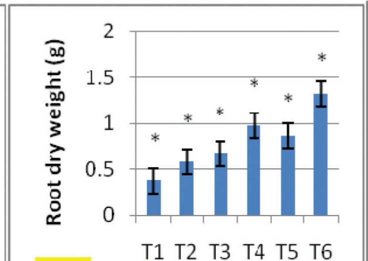

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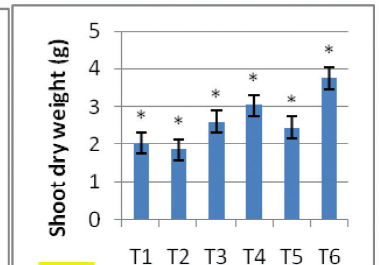

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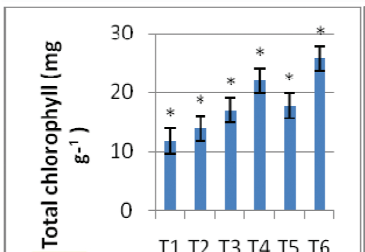

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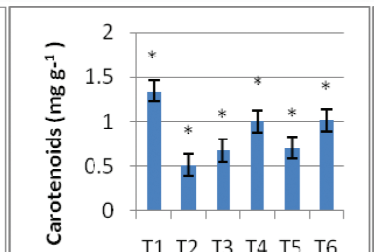

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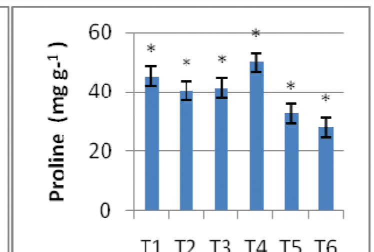

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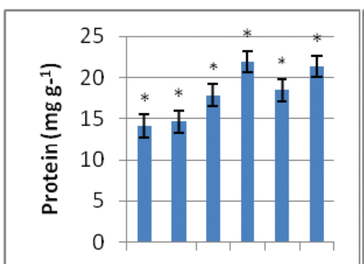

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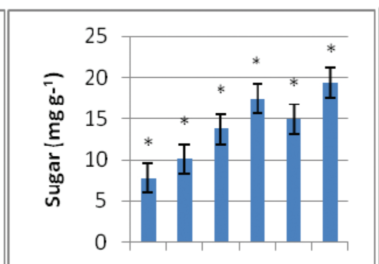

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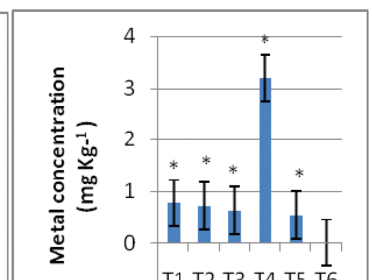

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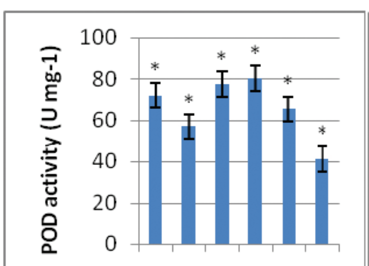

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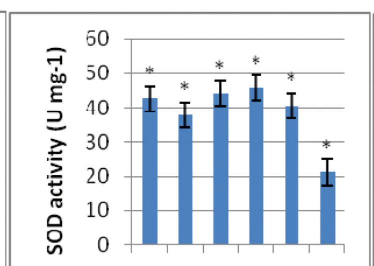

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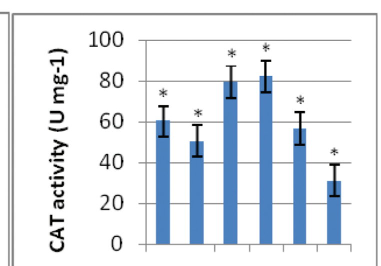

Fig:15

Set I: 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_1: 600 mg kg^{-1} soil (Fe at recommended levels); T_2: 600 mg kg^{-1} soil (Fe at recommended levels) + FYM@6t/h (Farm yard manure); T_3: 600 mg kg^{-1} soil (Fe at recommended levels) + Neem oil cake@4t/h; T_4: 600 mg kg^{-1} soil (Fe at recommended levels) + Mycorrhiza (*Glomus hoi*); T5: 600mg kg^{-1} soil (Fe at recommended levels) + Rhizobacteria consortia; T_6: Un-inoculated control (No treatment)

FYM was least effective (for Fe it was 14.65 mg g^{-1}; for Cr it was 14.07 mg g^{-1}) and Neem oil cake and Rhizobacterial amendments were better.

Activities of antioxidative enzymes in leaves of wheat subjected to metal treatments with and without AMF were assayed. The enzymes included superoxide dismutase (SOD), peroxidise (POD) and catalase (CAT). It was found that highest SOD activity was observed in AMF treated T4 plants (45.69U mg^{-1}), followed by Neem oil cake treated T3 (44.08U mg^{-1}), Rhizobacteria treated T5 (40.51U mg^{-1}), only metal treated T1 (42.59U mg^{-1}), FYM treated T2 (37.81U mg^{-1}) and T6 (42.59U mg^{-1}) which were un-inoculated control (No treatment). It was also found that highest POD activity was observed in AMF treated T4 plants (80.30U mg^{-1}), followed by Neem oil cake treated T3 (77.46U mg^{-1}), only Fe treated T1 (71.95U mg^{-1}), Rhizobacteria treated T5 (65.46U mg^{-1}), FYM treated T2 (56.90 U mg^{-1}) and T6 (41.25U mg^{-1}) which were un-inoculated control (No treatment). So, seeing this one can conclude that AMF was found to be most effective in enhancing SOD and POD activity as compared to other organic amendments. Similarly, to set I, here also AMF was found to extend POD activity to maximum and neem oil cake was found to be better than rhizobacteria, whereas FYM reduced POD concentration in host. These results are in accord with previous studies done. POD is responsible in the scavenging activity of superoxide ions (Hashem *et al.,* 2016). The SOD enzymatic activities of were reduced inmycorrhizal plants. These verdicts that AMF could shield plants by lessening cellular oxidative damage in response to Fe and Cr stress. The highest CAT activity was observed in AMF treated T4 plants (82.14U mg^{-1}), followed by Neem oil cake treated T3 (79.47U mg^{-1}), only Fe treated T1 (60.32U mg^{-1}), Rhizobacteria treated T5 (56.65U mg^{-1}), FYM treated T2 (50.44U mg^{-1}) and T6 (31.33U mg^{-1}) which were un-inoculated control (No treatment). Earlier studies by numerous investigators have established that AMF have the capacity to ratify antioxidants synthesis and upsurge their activity under metal stress. It has also been observed that the level of the antioxidant enzyme activities of GR, APX, CAT and SOD risesdue to cadmium (Garg and Kaur, 2013). The SOD, CAT and POD responses were associated with AM colonization and the induced activities of the enzymes by AMF contribute to the enhanced plant growth and metal tolerance of mycorrhizal associated plants under Fe and Cr stress.

CONCLUSION

The data reported in the present work clearly indicated that the symbiotic relationship between wheat and AMF can be established under heavy metal stress conditions, so one can utilize this beneficial property of AMF for bioremediation. The results showed that Fe and Cr have encouraged AM fungal colonization rate in the low heavy metal concentration in soil and reduced its value when heavy metal was used in high concentration (due to high level of plant toxicity). The facts and figures reported in the current work evidently indicated that the symbiotic relationship between host plant wheat and AMF- *Glomus hoi* can be established under heavy metal stress conditions, so one can utilize this beneficial property of AMF for bioremediation of heavy metals from soil with engineering industry effluent discharge. The inoculation of AMF also decreases heavy metal toxicity in plants. This is due to the AMF's ability for immobilizing heavy metal, which can reduce the translocation of heavy metal from soil to plant roots or for the ability of AM fungi to fixheavy metals in their cell wall, chelate them into cytoplasm sort them in their vacuole or restricting the influx of HM into the plant. Here host plant wheat was used, which belongs to grass family, hence shows better symbiotic association with AMF, produces good grains in spite of metal stress as AMF imparts tolerance to it and seeds usually do not store metal due to their natural physiology. Thus one can recommend use of AMF on host wheat for bioremediating sites at which industrial effluents are being discharged. This will not only reduce heavy metal concentration in soil reducing pollution, gives improved crop yield, but will also improve nutrient status of the soil.

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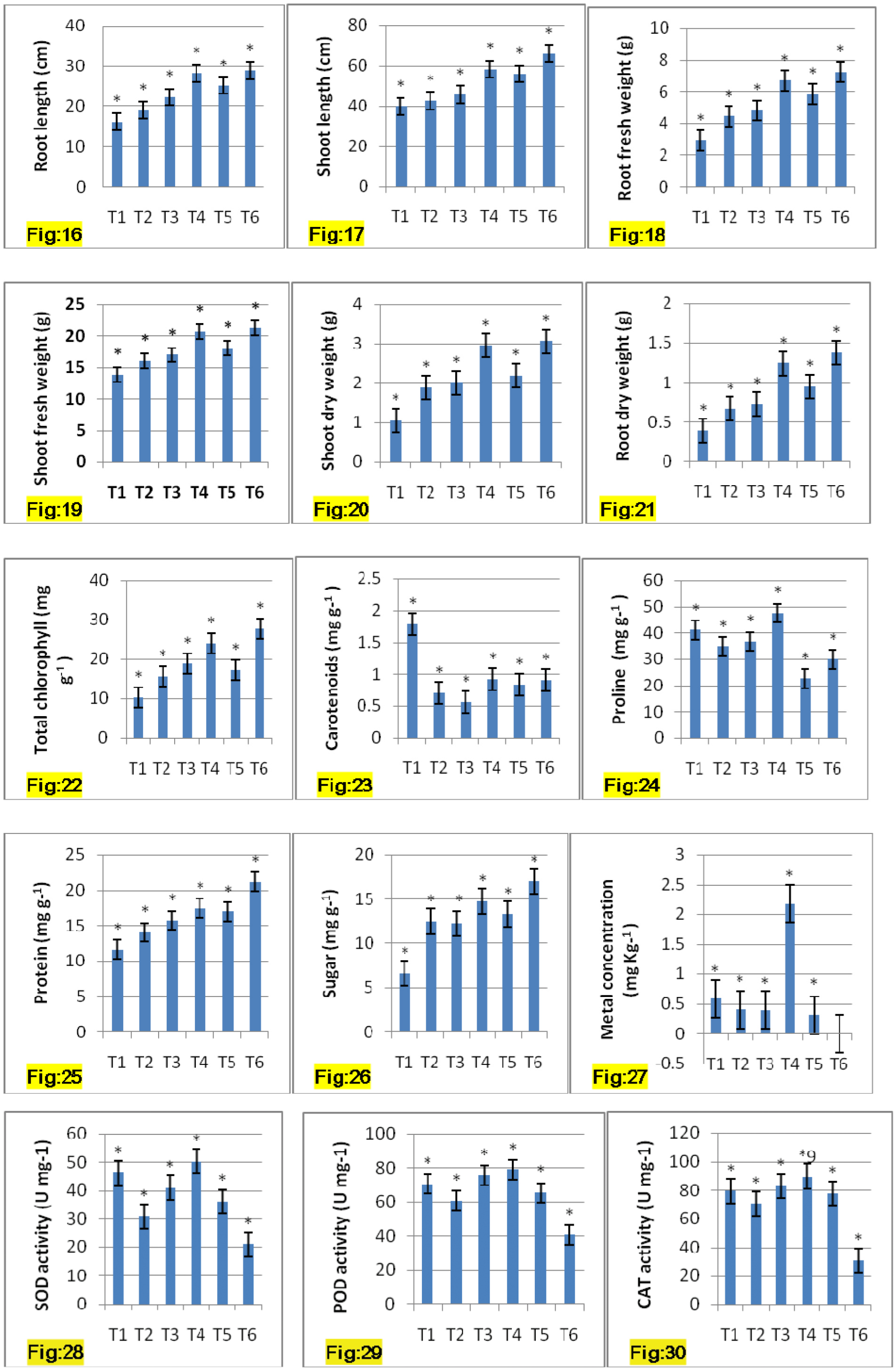

Set II: The data shown are the means ± standard error (n = 3). Value within each column marked with different * means values are * = significant and ** = not significant at p<0.05. Where, T: treatments, T1: 300 mg kg^{-1} soil (Cr at recommended levels); T2: 300mg kg-1 soil (Cr at recommended levels) + + FYM@6t/h (Farm yard manure); T3: 300 mg kg^{-1} soil (Cr at recommended levels) + Neem oil cake@4t/h; T4: 300 mg kg^{-1} soil (Cr at recommended levels) + Mycorrhiza (*Glomus hoi*); T5: 300mg kg-1 soil (Cr at recommended levels) + Rhizobacteria consortia; T6: Un-inoculated control (No treatment).

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