Journal of Bioresource Management

Volume 10 | Issue 2 Article 6

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Recommended Citation
Bilal, H., Raza, H., Sarfaraz, S., & Khan, D. H. (2023). Endophytic Potential of Entomopathogenic Fungi for the Remediation of Wastewater, *Journal of Bioresource Management, 10* (2).
ISSN: 2309-3854 online
(Received: Sep 10, 2022; Accepted: Nov 27, 2022; Published: Jun 30, 2023)

Endophytic Potential of Entomopathogenic Fungi for the Remediation of Wastewater

ENDOPHYTIC POTENTIAL OF ENTOMOPATHOGENIC FUNGI FOR THE REMEDIATION OF WASTEWATER

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ABSTRACT

Phytoremediation has the potential to significantly reduce water contamination caused by excessive harmful chemicals. The degradative properties of fungi are used in fungal phytoremediation to eliminate or neutralise the hazardous pollutants present in water. The goal of the current study was to endophytize water lettuce with the two entomopathogenic fungus *Metarhizium anisopilae* and *Trichoderma harazium*. The plant is inoculated with the fungus using the root-dipping procedure. There were two main treatments and a control all with five replications. The analysis of plant and wastewater were analyzed initially like frequency of fungus remained in plant weight, root length and for water that was Biological oxygen demand (BOD), Chemical Oxygen demand (COD), and heavy metals (Copper, Nickle, Zinc and Cadmium). The data were taken for 3rd 5th and 7th day of the experiment. The results exhibit that *T. harazium* exhibited the 82.67 % followed by the *M. anisopilae* with 65.33 % as compared to control with 1.33 % mean frequency the 10th day of inoculation. Maximum weight 295.98 and 265.13 g and root length were maximum recorded 15.18 and 18.12 cm respectively at the end of the experiment. Performance of *T. harazium* endophytic plant found to be 90.7 % for BOD, 73.82 % for COD. The removal % of Cu, Zn, Ni, Cd exhibited 75.13, 96.58, 87.14, 61.17 % after 7d of treatment. In case of M. *anisopilae,* 85.2 % for BOD, 69.38 % for COD. The removal % of Cu, Zn, Ni, Cd exhibited 66.48, 89.43, 77.42, 52.4 % after 7d of treatment. The treatments exhibited the remarkable reduction in pollutants and increase in plant weight and root length.

Keywords: Phytoremediation, myco-remediation, water lettuce, wastewater, contaminant.

INTRODUCTION

Phytoremediation is a methodology or procedure that uses naturally occurring or genetically engineered plants to remove, transfer, stabilize, or minimize different toxins found in soil or water (Yan et al., 2020; Nedjimi, 2021). A phytoremediation technique for wastewater treatment has a number of benefits, including being economical, requiring little energy, causing less environmental disruption, etc (Abdul Aziz et al., 2020). It has been demonstrated that using plants to recover water that was contaminated with heavy metals is successful (Ali et al., 2020).

Bioremediation also known as mycoremediation employs the degradative properties of fungi to eliminate or mitigate the toxic pollutants that are present in soil and water (Kumar et al., 2019). This process is developing as an effective technique for effluent treatment and being increasingly adopted (Nandy et al., 2020). As endophytic metabolites offer a wide range of adaptations to host plants boosted by enhancing plant resilience to biotic and abiotic stressors, as well as improving plant development, endophytic fungi can both actively and passively stimulate plant growth through a wide range of benefits (Kaur, 2020). In contrast to the untreated plants, the endophytic plants exhibited increased biomass in the shoots and roots

(Shymanovich and Faeth, 2019). Endophytic fungi can perform a direct or indirect function in the phytoremediation process and degradation of the toxic elements. They can accomplish this indirectly by promoting the growth of plants with phytoremediation abilities, which speeds up the whole process, and otherwise directly by degrading and/or accumulating contaminants by themselves (Sudha et al., 2016).

Trichoderma species, which are particularly prevalent in nature and have the potential to colonize widely various environments, are among the fungus that can be utilized in the bioremediation (Kacprzak et al., 2014). There are reports that *Trichoderma* can protect plants against stress factors, particularly infectious microorganisms, and boost plant development by up to 300 % (Zin and Badaluddin, 2020; Tyśkiewicz et al., 2022). *Trichoderma* fungi are known for their potent fungi that are aggressive against diseases and their capacity to alter the rhizosphere microbiota of plants through vigorous root colonization (Kacprzak et al., 2014; Tyśkiewicz et al., 2022). In case of Metarhizium, it supports the host plant by increasing stress resistance (Dara, 2019), increasing plant biomass and growth (Barelli et al., 2020), stimulating root growth (Siqueira et al., 2020), and antagonizing plant diseases (Hummadi et al., 2022). It is reported that plant roots treated with Metarhizium developed more quickly and had a higher density of root hairs when compared to control plants (Sasan and Bidochka, 2012). These beneficent fungi are among the species that are most resistant to toxins and chemicals, whether they are natural or manmade, and they can even suppress some of them (St. Leger and Wang, 2020).

The goal of this study was to compare the water quality prior to and after endophytic water lettuce-based phytoremediation in order to assess the efficacy of two entomopathogenic fungi, *Trichoderma harazium* and *Metarhizium anisopilae*. Due to their quick development rate and adaptability of a range of environmental conditions, these microorganisms were chosen as the phytoremediation agents in this investigation.

MATERIAL AND METHODS

Sampling and Laboratory Analysis

Samples of the sewage effluent were taken in Multan, Pakistan at the wali Muhammad distributary. pH, Electrical conductivity, TSS, COD, BOD, and Heavy Metals were the initial data parameters analysed in the laboratory. For each parameter, three readings were taken. Before being used for the experiment, the sewage effluent samples were filtered to get rid of any unnecessary materials.

Fungal Isolate

The fungal strains studied were *T. harazium* and *M. anisopilae*, which were taken from the MNS-University of Agriculture in Multan. After being incubated for 10 days at 25°C in complete darkness, cultures grown on potato dextrose agar media yielded conidial. In test tubes containing 0.01 % (v/v) Tween 80, conidia were collected. After cell counting with a Neubauer hemocytometer, suspensions were vortexed for 2 min, filtered through four layers of sterile muslin, and adjusted to 1×10^8 conidia ml^{-1} (Gurulingappa et al., 2010). Before each experiment, conidial viability was evaluated (Goettel et al., 2000). In order to ensure the consistency of the viability ratings, this germination test was repeated for each suspension of a stock. The conidia's average viability was higher than 95 % in every case.

Inoculation Technique

In this research, water lettuce was chosen as a phyto-remedial agent. The plant samples were thoroughly rinsed, particularly around the roots, to remove any foreign materials before the trail, and they were then placed in a tank with distilled water for a week to neutralise the plants. For fungal inoculation, roots were submerged in solution. For better absorption, the roots' ends were clipped, and each root was inserted in a test tube with 2 ml of a conidial suspension (1×10^8 conidia ml^{-1}) (Akello et al., 2007). Control plants had their roots immersed in sterile, distilled water. Both the control and treated plants were dried fully on sterile filter paper. Each treatment has five replicates, ten plants in each container and total fifteen containers with measurements of 38 cm × 25 cm × 15 cm for treatment were used. Each plant weighed 30 g, and six litres of sewage wastewater were placed in the container with the plants.

Evaluation of Fungus in Plant

To investigate the prevalence of fungus in plant tissues, one leaflet from each plant was randomly chosen then surface-disinfected by immersion in 0.5 % sodium hypochlorite for 2 minutes, followed by 120 seconds in 70 % ethanol and rinsed using sterile distilled water. In a laminar flow cabinet, the leaves were dried on sterile paper towels before having the corners chopped off to remove any dead tissue that resulted from the sterilisation procedure. The disinfection of the leaves was confirmed by plating 100 ml of the last rinsing water from each sample onto Potato dextrose agar (PDA) (Gurulingappa et al., 2010).

Using a sterile scalpel, leaves were divided into five parts, each measuring around 1 cm^2. Five leaf segments from each of the treated and untreated (control) plants. A 0.1 % stock was made by dissolving 0.02 g of each antibiotic (tetracycline, streptomycin, and penicillin) in 10 ml of sterile distilled water. This stock was then sterilised through a 0.2 m filter paper before being added to each litre of medium at a ratio of 1 ml (Vega et al. 2008).

Leaf segments were put on plates that had PDA and antibiotics in them. The three duplicates of each endophytic plant treated and control plants. We looked at 450 leaf parts from a total of 150 plants. After 10 days at 25°C, the fungus' presence or absence on the leaf sections was noted. The number of colonised leaf sections divided by the total number of leaf segments was used to express the data (Petrini and Fisher 1986).

Plant Parameters

The plants' root length (cm) was measured directly with a 30 cm calibrated scale. A electronic weighing balance was used to measure the fresh weight of the plants after they had been air dried.

Data Analysis

One way ANOVA test using the Statistica 8.1 was performed in this study to determine the significant differences between the parameters of sewage wastewater quality before and after.

RESULT AND DISCUSSION

The removal of metals from contaminated water via bioremediation has received widespread acceptance as an environmentally benign technique. The bioremediation capacity of two endophytic fungi inoculated in water lettuce was evaluated in the current study for TSS, BOD, COD, and heavy metal (Zn, Cu, Ni, and Cd).

We evaluated the frequency of endophytic colonization of water lettuce plants by *T. harazium* and *M. anisopliae* according to post-inoculation time (10 days). Result exhibited trend in following order; T.h > M.a > Control with the *Trichoderma harazium* exhibited the highest mean frequency 82.67 % followed by the *M. anisopilae* with 65.33 % as compared to control with 1.33 % mean frequency the 10th day of inoculation.

a

b

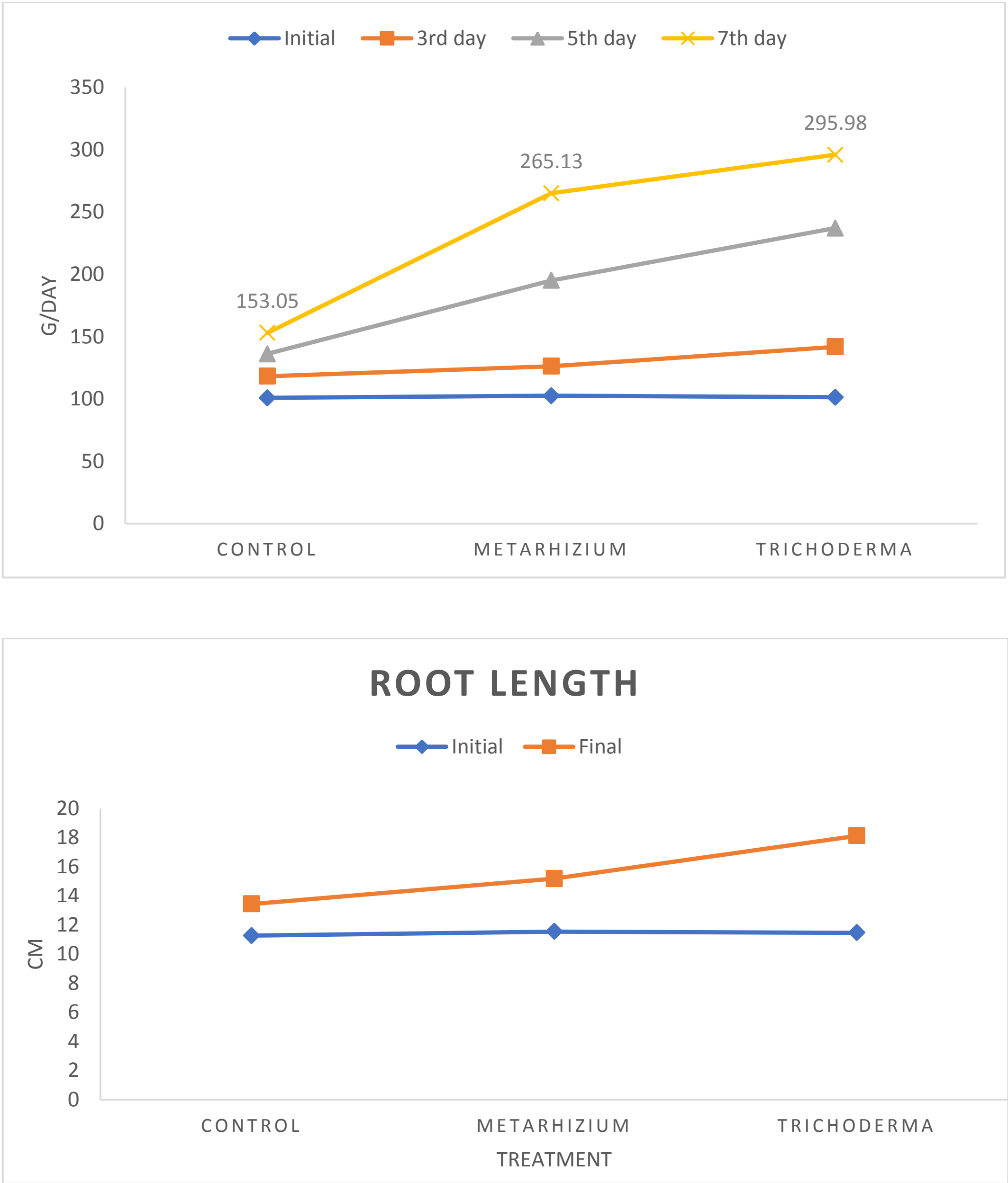


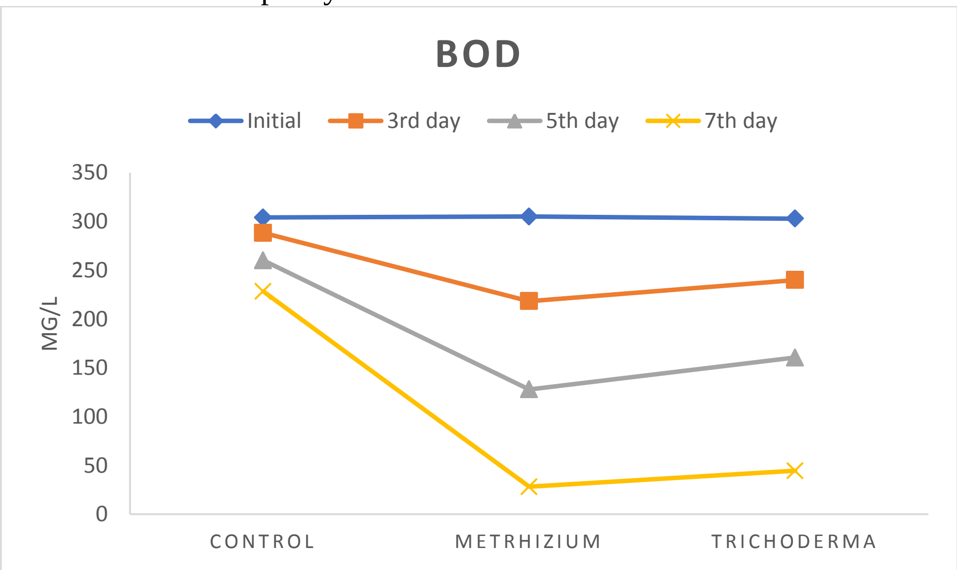
Figure 1: It represents the mean a: plant weight and b: root length of endophytic and control group of water lettuce. The graphs demonstrate the effectiveness of Metarhizium and Trichhoderma as a growth regulator.

The plant weight and root length were assessed before and after the 3rd, 5th and 7th day. The remarkably increase weight and root length was observed in the case of *T. harazium* endophytic water lettuce plant. There was significant variation among the *T. harazium (T.h)* and *M. anisopilae (M.a)* endophytic plant for plant growth when compared with the control plant. The mean weight and root length of water lettuce was found in the following order; T.h > *M.a* > control after 3d, 5d, and 7d of treatment. Maximum weight of 141.88, 237.12, 295.98 g was observed respectively in water lettuce endophytic with *T. harazium. M. anisopilae* endophytic in water lettuce exhibited maximum weight of 126.32, 195.13, 265.13 g at 3d, 5d, and 7d respectively Fig1,a. Root length maximum recorded at 7 days 15.18 cm and 18.12 cm respectively Fig1,b. Trichoderma spp.

encourages plant growth by a number of processes, including raising the production of vitamins (Prisa, 2020), improving nutrient absorption and translocation (Sood et al., 2020), accelerating root development (Zin and Badaluddin, 2020), and speeding up the pace of carbohydrate metabolisms and photosynthesis (Şesan et al., 2020).

A few particular Trichoderma species have the capacity to create phytohormones like auxin and auxin-like secondary metabolites (Illescas et al., 2021), gibberellic acid (GA_3) (Jaroszuk-Ściseł et al., 2019), as well as to change the relationship between the hormones cytokinin and auxin. According to a study, the evaluation of 106 Trichoderma isolates exhibited that 60 % of the isolates were able to produce IAA and auxin analogues (Hoyos-Carvajal et al., 2008).

a



b

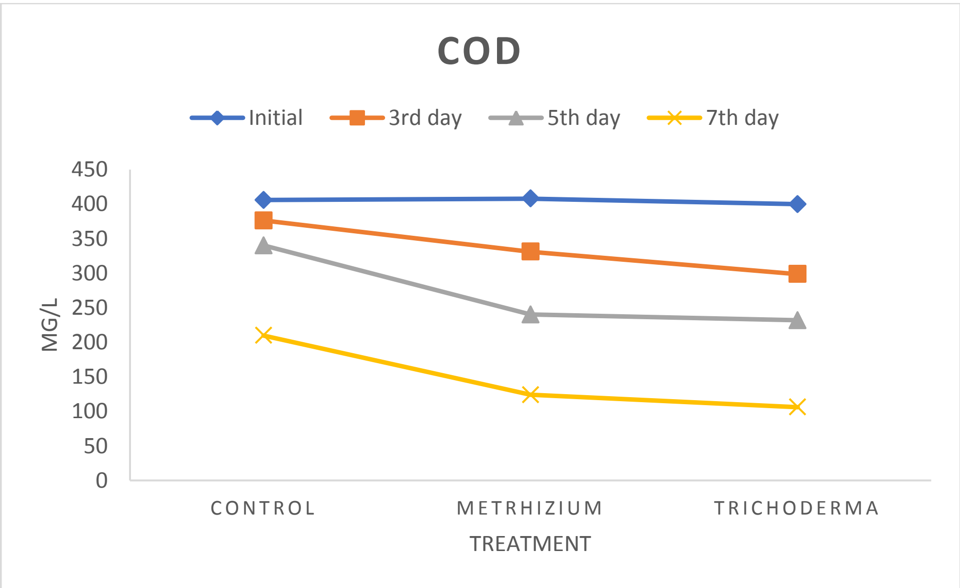


Figure 2: It represents the mean a: BOD and b: COD of endophytic and control group of water lettuce. The graphs demonstrate the remediation potential of endophytic fungi Metarhizium and Trichhoderma in the water lettuce.

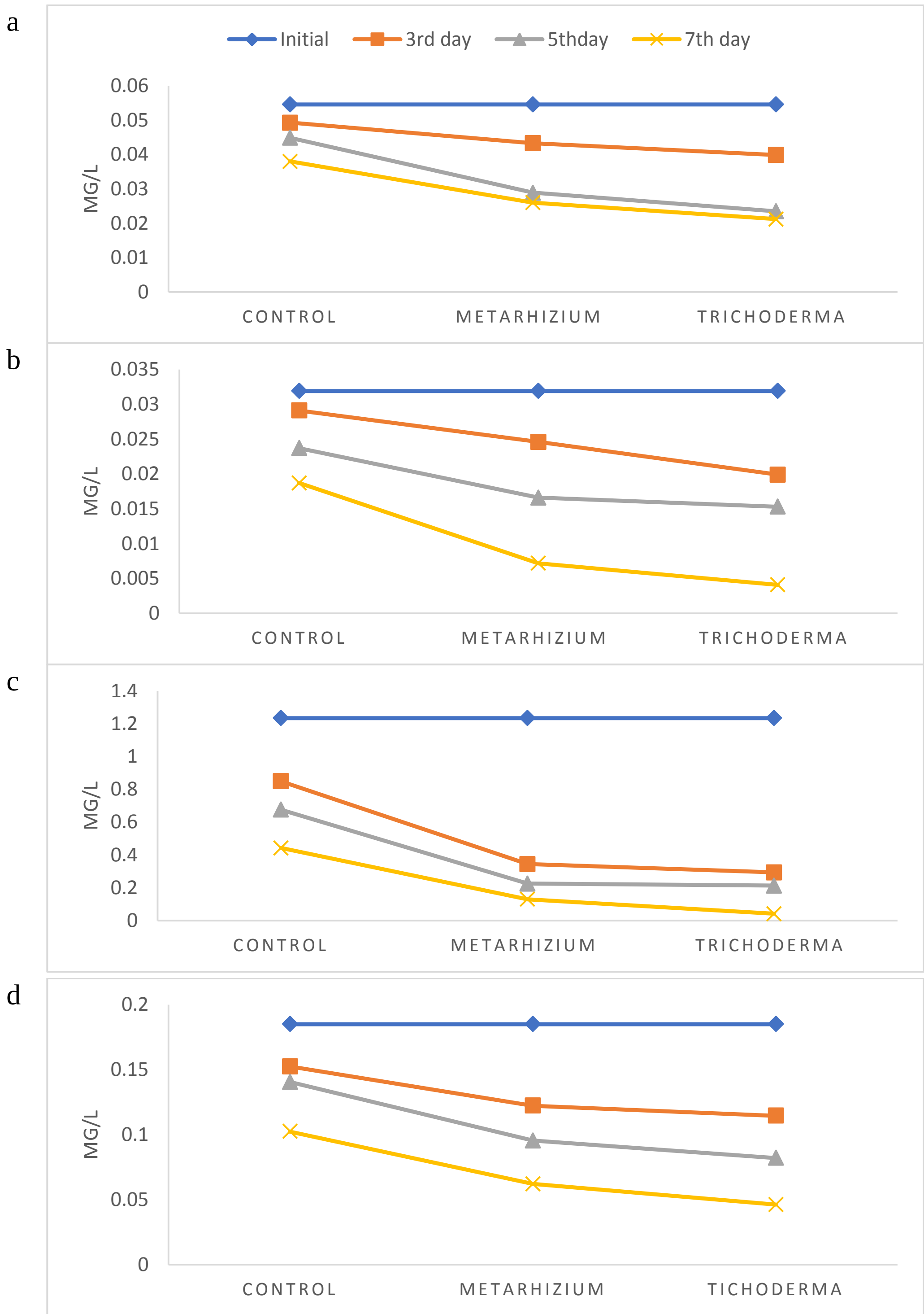


Figure 3: Graphs represents the mean uptake of following heavy metals a: Cadmium, b: Nickle, c: Zinc and d: Copper from waste water. These demonstrates the remediation potential of endophytic fungi Metarhizium and Trichhoderma in the water lettuce against control group.

By creating IAA and other substances that encourage plant growth, endophytic fungus have the capacity to enhance growth and remediation of habitats that are heavily metal contaminated (Hoseinzadeh et al., 2017).Phytoremediation treatment of sewage wastewater using water lettuce with two entomopathogenic fungi showed a massive decrease in BOD and COD values (Figure 3,4). The average initial reading for BOD and COD was 304 and 405 mg/L respectively before the treatment. The values was maximum decreased by water lettuce endophytic with *T. harazium* were 28.2 and 106.2 mg/L at the 7^{th} day of the treatment. In case of endophytic *M. anisopilae* the reduction was 44.6 and 124 mg/L at the end of the treatment respectively. Water lettuce had an average removal effectiveness of 33.43 % for BOD and 26.37 % for COD, according to (Shah et al., 2014). The increased organic matter clearance by microorganisms in sediments or on the surfaces of plant rootstocks was linked to the sedimentation of suspended particles and the transfer of oxygen to the root surface by plants. (El-Din and Abdel-Aziz, 2018).

The heavy metal uptake by the water lettuce endophyte with *Metarhizium* and *Trichoderma* were determined. The objective was to improve the heavy metal removal uptake of water lettuce from the sewerage wastewater. The removal % of Cu, Zn, Ni, Cd by water lettuce colonized with *M. anisopliae* exhibited 66.48, 89.43, 77.42, 52.4 % respectively after 7d of treatment. The removal % of Cu, Zn, Ni, Cd by water lettuce colonized with *T. harazium* exhibited 75.13, 96.58, 87.14, 61.17 % after 7d of treatment (Figure 4). The buildup of heavy metals in plants is entirely supported by the results of our research. Through a variety of methods, including phyto-extraction, phyto-stabilization, phyto-volatilization, and rizhofiltration, heavy metal accumulation in plants can be remedied. In these methods, plants absorb heavy metals from their roots, and these HMs then migrate toward the plants' shoots and leaves. Due to their high level of resistance to certain metals, plants can be grown.

CONCLUSION

As far Plant growth are concerned *T. harazium* found most effective while considerable increase by the *M. anisopilae* endophytic potential in water lettuce. In case of removals of pollutants were also found with the former fungi. Performance of *T. harazium* endophytic plant found to be 90.7 % for BOD, 73.82 % for COD. The removal % of Cu, Zn, Ni, Cd by water lettuce colonized with *T. harazium* exhibited 75.13, 96.58, 87.14, 61.17 % after 7d of treatment. The system's processes for pollutant removal include chemical transformations, sorption, sedimentation, volatilization, and both aerobic and anaerobic microbiological conversions. It could be beneficial to pre-treat wastewater before the facility becomes acclimated. By further examining the removal effectiveness under a variety of various environmental conditions, the option of using macrophytes for the treatment of municipal sewage under local environmental conditions can be explored. Additionally, it is imperative that macrophyte systems be employed to treat wastewater because of their comparable performance to that of traditional wastewater treatment facilities and their extremely low O&M costs.

AUTHORS CONTRIBUTION

The research paper involved the collaborative efforts of all the authors. Huda Bilal and Hasnian Raza played pivotal roles in shaping the project, making significant contributions throughout the research process. Huda's expertise and dedication enriched the literature review, data collection, and analysis. Hasnian's meticulous approach ensured the accuracy and reliability of the collected data. Sana Sarfarz and Danyal Haider also made valuable contributions to the study, actively participating in the research design, data collection, and manuscript preparation.

CONFLICT OF INTEREST

The authors have no conflict of interest

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