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PERFORMANCE OF TRITICUM AESTIVUM L. (WHEAT) UNDER THE INFLUENCE OF FUNGAL INOCULATION TO SUSTAIN ARSENIC STRESS

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ABSTRACT

Wheat is one of the most important staple foods worldwide, dominating other crops in terms of acreage and production. However, wheat is highly vulnerable to the toxic effects of heavy metals such as cadmium, lead, mercury, and arsenic. Arsenic (As), a trace toxic metalloid, poses significant environmental challenges, severely impacting water, soil, animals, and humans. In plants, arsenic causes both physiological and morphological damage by inducing the generation of reactive oxygen species (ROS), which in turn harm nucleic acids, proteins, and membrane lipids. In this study, two wheat varieties—Dilkash and Fakhr-e-Bakkhar—were inoculated with the endophytic fungus *Fusarium oxysporum* to mitigate Arsenic stress. This fungus forms a mutualistic association with plant roots, expanding the rhizosphere surface area without harming plant tissues or cells. Arsenic concentrations in wheat tissues followed the order: roots > shoots > leaves > grains. Grain arsenic uptake showed a positive correlation with both available arsenic in soil ($r = 0.678$, $p < .0001$) and total soil arsenic content ($r = 0.23$, $p < .0001$). Application of *Fusarium oxysporum* at 5 g kg^{-1} soil significantly ($p < .004$) reduced arsenic levels in grains across all treatments. Moreover, grain arsenic content was negatively correlated with total soil glomalin ($r = –0.320$, $p < .004$), fungal colonization ($r = –0.115$), and soil phosphorus content ($r = –0.762$, $p < .0001$). Overall, the findings demonstrate that inoculation with endophytic fungi can effectively lower arsenic accumulation in different parts of wheat plants grown in arsenic-contaminated soils.

Keywords:

INTRODUCTION

Wheat is an important crop that belongs to Family poaceae takes up the most farmed land around the world. Many growth-limiting factors prevent wheat from producing a high yield. Numerous chemicals have entered the environment as a result of modern agriculture, urbanization, and increased global industry. The metals cannot break down like biological materials and must often be manually removed or immobilized makes it a challenging process (Malik et al., 2020). When metals infiltrate the food chain, contaminate groundwater aquifers, and come into contact with contaminated soil, they disrupt ecosystems and have a negative impact on human health. This pollution decreases cultivable and fertile ground for farming, as well as productivity and food quality. The rate at which greenhouse gas emissions are causing air pollution is rising. Heavy metals (HMs) or trace metals, such as arsenic (As), aluminum (Al), cadmium (Cd), chromium (Cr), beryllium (Be), mercury (Hg), copper (Cu), lead (Pb), iron (Fe), nickel (Ni), zinc (Zn), and thallium (Tl), are released into the atmosphere by anthropogenic activities, disrupt the function of essential cellular components, contaminate groundwater, and pose a health risk (Riseh et al., 2023). These metals also interfere with the level of antioxidants in plants and reduce the nutritional value of the product.

Arsenic is harmful metal that plays important role in the environmental resources such as natural geochemical processes and

anthropogenic activities (Mandal et al., 2002). The International Agency for Research on Cancer (IARC) and US Environmental Protection Agency (EPA) have ranked As and its compounds as a Group 1 human carcinogen (Rosas-Castor et al., 2014) (Niazi et al., 2018). The Agency for Contaminated Substances and Disease Registry (ATSDR) has ranked Arsenic at the top among the 20 priority harmful substances (Abbas et al., 2018). It is mostly found in the environment as oxyanions of pentavalent arsenate As (V) or trivalent arsenate As (III) being more hazardous to biological systems than As (V) (Smedley et al., 2002). Arsenic (As) is present in the groundwater and soil as a pollutant. It is a non-essential element for plants and found in two main inorganic phytoavailable forms i.e. arenite (AsIII) and arsenate. Arsenic can also move to other plant tissues, discoloring and plasmolyzing the root, inhibiting the production of chlorophyll and reducing its capacity for photosynthetic activity Therefore, it essential to adapt methods to reduce metal toxicity in plants and their rhizosphere (Soto et al., 2019).

Growth-promoting endophytes play an important role in the alleviation of abiotic stress in plants improving host tolerance to hostile environments, such as high heat, salinity, drought, cold and heavy metal stress (Afzal et al., 2014). Phytoremediation is a part of bioremediation where existing plants and plants inhabiting microbes are used to clean pollutants from the environment. These contaminated chemicals include carcinogenic agents, metals and metalloid pollutants, inorganic pesticides and herbicides, chlorinated products, industrial organic waste material and excess radionuclides and nutrients (Deng et al., 2017).

Certain root endophytic fungi carry many aids to plants, such as stimulating plant nutrient absorption, increasing plant resistance to severe environments (such as salt, pH, metal stresses and temperature) thus increasing crop production (Nasif et al., 2023). Abaya et al., (2021) demonstrated that 90 endophytic fungi from two different wheat varieties and estimated their ability to stop the growth of wheat pathogens, i.e., Fusarium graminearum and Waitea circinate. El-Shahir et al., 2021 reported that impact of endophytic *T. pinophilus* on the growth of *T. aestivum* grown in heavy metal-contaminated soil. Here we studied the role of *Fusarium oxyporium* to improve heavy metal stress tolerance in wheat through biochemical and molecular approaches under Arsenic stress.

MATERIAL AND METHODS

All experiments were conducted with four replicates and 10 seeds will be sown per pot. This experiment was conducted in the Botanical garden of Lahore College of Women University, Lahore, Pakistan.

Soil samples were collected from the bottom most horizon at Ravi river, Lahore and Kaala Shah Kaaku industrial area district Sheikhupura, Pakistan. Cleaned the soil with sieve and transfered to a polythene bags and label it. Formation of soil solutions was carried out according to the Tipping et al., 2003.

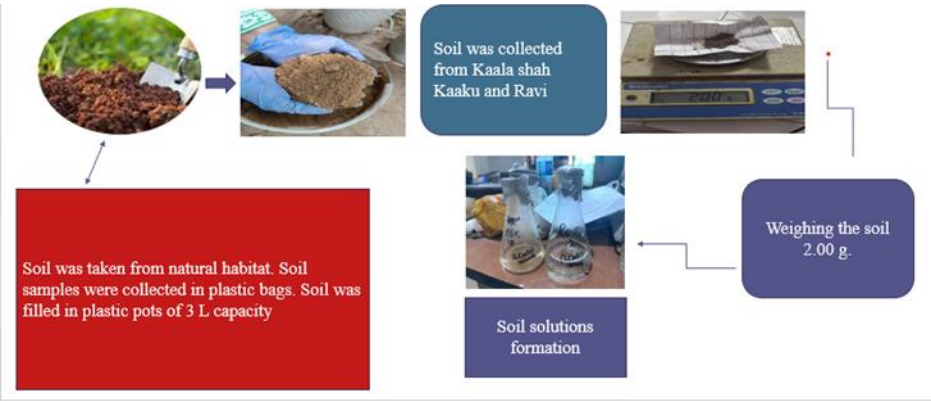

Figure 1: Formation of soil solutions.

Seeds of two wheat varieties (Dilkash and Fakhr-e-Bakhar) obtained from Federal Seeds Corporation, Lahore, Pakistan; labelled them and cleaned the grains with the help of sieve size 2nm (Ohm et al., 2013).

Fungal isolation

A sterile surgical blade was used to cut the samples and incorporated into potato dextrose agar (PDA) media plates and sealed them with paraffin tape as well as labeled them too. The whole process of isolation was taken place into complete sterilized environment of laminar air flow as showed in figure 2. After that labeled petri dishes put into incubator with temperature set up of 25±2°C and left them for a week of incubation period. After this period a handsome growth of *F. oxyporium* was showed into plates which were confirmed after microscope examination. Further cultures were purified with the help of these pure cultures for future use respectively. Fungus expressed visible white cotton like growth surrounding during the inoculation (Priyadharsin et al., 2017). All the samples collected into plastic vials and saved on room temperature for future application in fungal isolation. After incubation of 7 days, a handsome growth of (*Fusarium oxyporium*) was seen into plates which was confirmed after microscope examination. Further cultures were purified with the help of these pure cultures for future use respectively (Abdalla et al., 2014). The colony characters were studied by growing the isolate at 28 °C on PDA for 7 days (Fisher et al., 1982). The surface of petri plates was sterilized with 2% hydrogen peroxide for 1 min and 0.1% mercuric chloride for 30 sec. These steps were repeated three times. After the confirmation that plates were free of bacterial contamination they were inoculated with the isolation. For microscopic study from the PDA plates small portions of the fungal sample. The pure culture was obtained from subcultures and maintained on PDA plates. The formation of colonies was initially recognized by morphological appearance that was white cottony on the under surface of PDA plates. Slides were prepared and microscopic identification was checked which showed the formation of septate mycelium and sickle shaped macro and micro conidia. This leads to the confirmation of the *F. oxyporium* (Sharma et al., 2024).

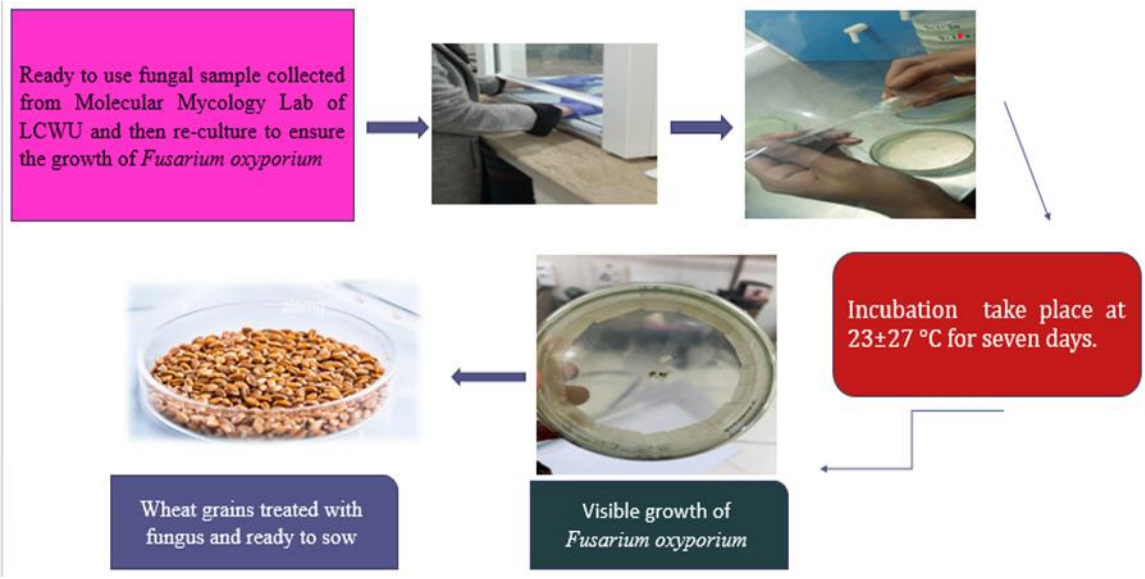

Figure 2: Isolation and preservation of Fusarium oxyporium.

Pot experiments to check the performance of wheat (Triticum aestivum) under the influence of endophytic fungus (Fusarium oxyporium) and heavy metal (Arsenic) stress

The pot experiment started with the seed sowing. The pot size was 9cm in width and 10cm in height. Potting mixture contained 2-3 kg soil as well as inoculation of endophytic fungus (*Fusarium oxyporium*) which were applied for few mins according to treatment plan. Before sowing the seeds and for metal accumulation solution of arsenic oxide 12 mg/L was added. Fungal culture prepared by using PDA media (potato dextrose agar media) with concentration of 39 g/1000 ml and incubated into incubator at 25◦C respectively. A week-old fungal culture was used to scraping colonies with the help of spatula and then flooded out media plates with sterilized water. Then fungal suspension was filter through 4-layer cheesecloth (50 µm) which control mycelia and then after that the conidia concentrations adjusting according to (1×106 conidia ml–1) with the help of hemocytometer (Zheng et al., 2013). After two days of fungal inoculation, sterilized water was applied to plants which maintained humidity at ~70% for the early blight progression. Experiment took place with 4 replicates of every treatment into complete randomized design (CRD). With experimental pots, some pots are arranged as control groups because they receive only water. There are the treatment plans for pot experiment.

Variety 1 = V1 = Dilkash

Variety 2 = V2 = Fakhr-e-Bakhar

T1 = Fungus inoculation (Fusarium oxyporium)

T2 = Heavy metal (Arsenic) Accumulation

T3 = Fungus (Fusarium oxyporium) Inoculation + heavy metal (arsenic) accumulation

T4 = Control with no amendment

Figure 3: Pot experiments to check the performance of wheat (Triticum aestivum) under the influence of endophytic fungus (Fusarium oxyporium) and heavy metal (Arsenic) stress.

Conformation of endophytic fungus

Roots are dried and kept for re-hydration for 1-2 hours in water. Roots were washed with tap water on sieve size 2nm .After washing the root parts with tap water, the roots were soaked at 1% KOH overnight. On next day, KOH pour off and then washed the roots with tap water for 2-3 times. Then roots were acidified with 1% HCL for the 10 minutes at room temperature. This step was compulsory for the trypan blue or congo red stain to bind fungal structures shown in figure 4. Then slides are prepared by using this stain which provide cleared Endophytic fungus identification confirmed by the microscopy. Small portions of root cut in with blade and then put into slides to noticed the growth of endophytic fungus in wheat plant (Suwandi et al., 2012).

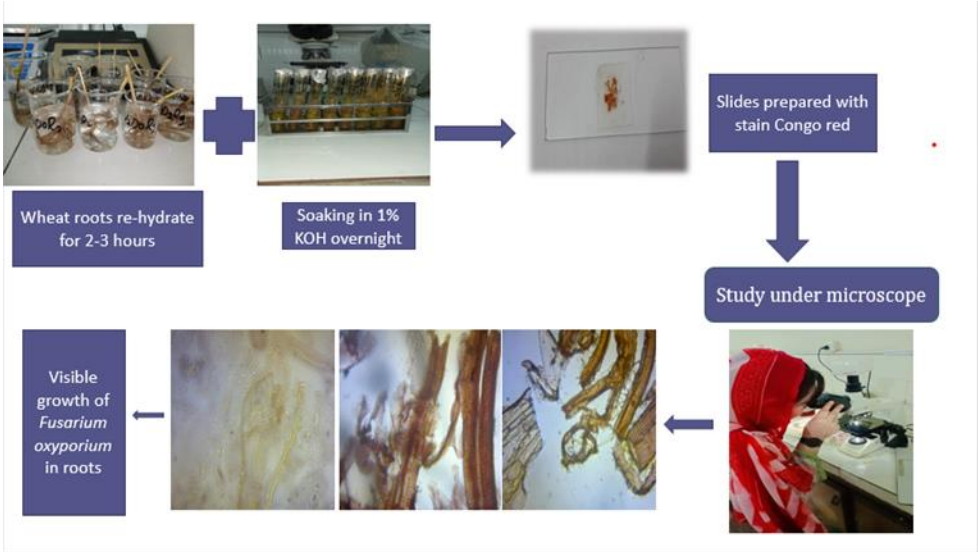

Figure 4: Fungal growth in the roots of wheat plants.

Chlorophyll content estimation

Chlorophyll content was measured in wheat leaves in accordance with the method of Hiscox and Israelstam (Babu et al., 2015) using DMSO (Dimethyl Sulfoxide) after 45 and 60 days respectively. In 7 ml DMSO add 100 mg fresh leaf samples and incubates them for 50 minutes at 65◦C. Now supernatant decants and the leaf tissues discarded as well. Then add DMSO into supernatant and make the final volume up to 10ml. By considering DMSO as a blank, take extract absorbance reading at 645, 475 and 663nm. With the help of this formula total chlorophyll contents were calculated from the given sample. Formulas for the quantification of chlorophyll a, b and carotenoids is given below respectively.

Chl a (mg g–1 f. wt.) = (12.7×OD663–2.69×OD645) × V/W/1000

Chl b (mg g–1 f. wt) = (22.9×OD645–4.68×OD663) × V/W/1000

Total chlorophyll content = [20.0 (A645) + 8.02 (A663)] × [V/100 ×W× a]

Carotenoids (mg g–1 f. wt) = (Acar /EM) × 1000

EM = 2500

A: Absorbance at specific wave length of 645, 475 and 663 nm

V: Final volume of the chlorophyll in extract (ml),

W: Fresh weight of the sample (g)

a: Path length of light (cm)

Sample digestion for arsenic analysis

Wheat samples 0.2-0.5g taken into clean dry digestion tubes. Added the 5ml of concentrated HNO3 into it. Mixture allowed to stand overnight under fume hood. On following day test tubes were placed in a heating block 0n 60 ◦C for 2 hours. Test tubes were then allowed to cool at room temperature then 2ml concentrated $HCLO_4$ added to plant samples (Cerveira et al., 2020) Tubes heated at 160 ◦C for 4 hours. Heating stopped when dense white fume of HCLO4 emitted. The content was allowed to cool added the diluted 25ml with distilled water and filtered through Whatsman's No. 1 filter paper for separation of plant samples and finally store in plastic vials. All glass goods washed with 2% HNO_3 followed by rinsing with de-ionized water and then dried.

RESULTS

Root length (cm)

ANOVA was used for the measurement of root length of plant, the results shown in figure 5. The fungus plus arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant root length means and figure 5 provides a graphical representation of same data. The root length of V2 showed higher growth at the fungus + arsenic treatment.

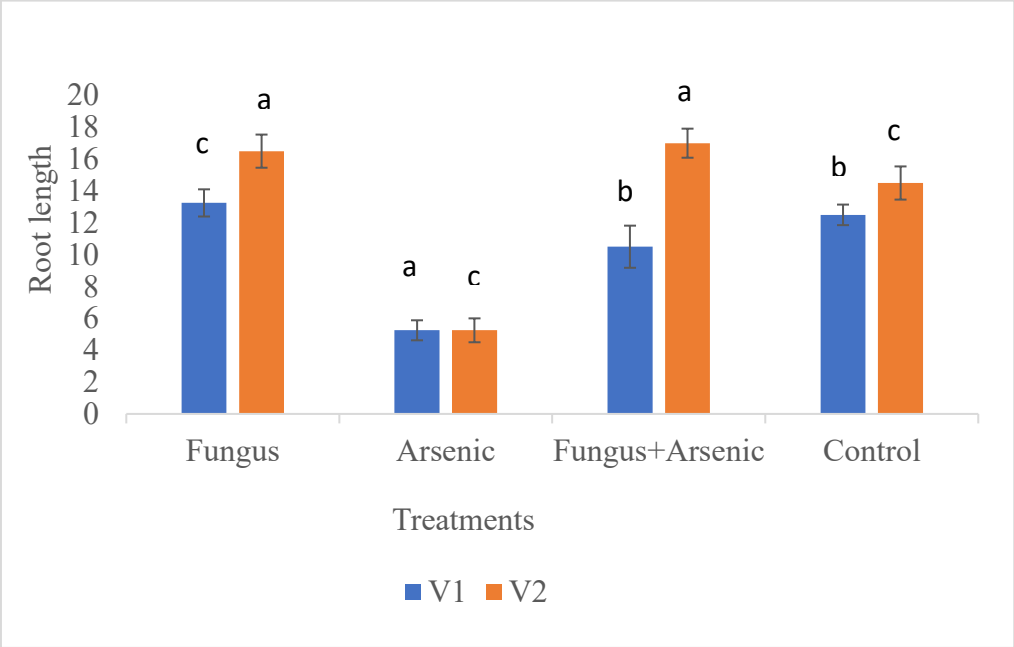

Figure 5: Length of wheat roots grown in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

Shoot length (cm)

ANOVA was used for the measurement of shoot length of plant (Figure 6). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Maximum growth was showed by V2 at fungus + arsenic treatment.

Number of leaves (cm)

ANOVA was used for the measurement of number of leaves of wheat (Figure 7). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Number of leaves with higher growth at V2 by Fungus + arsenic treatment.

Dry weight of leaves (g)

ANOVA was used for the measurement of dry weight of leaves (Figure 8). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Maximum leaves growth was shown by arsenic fungus treatment of V2.

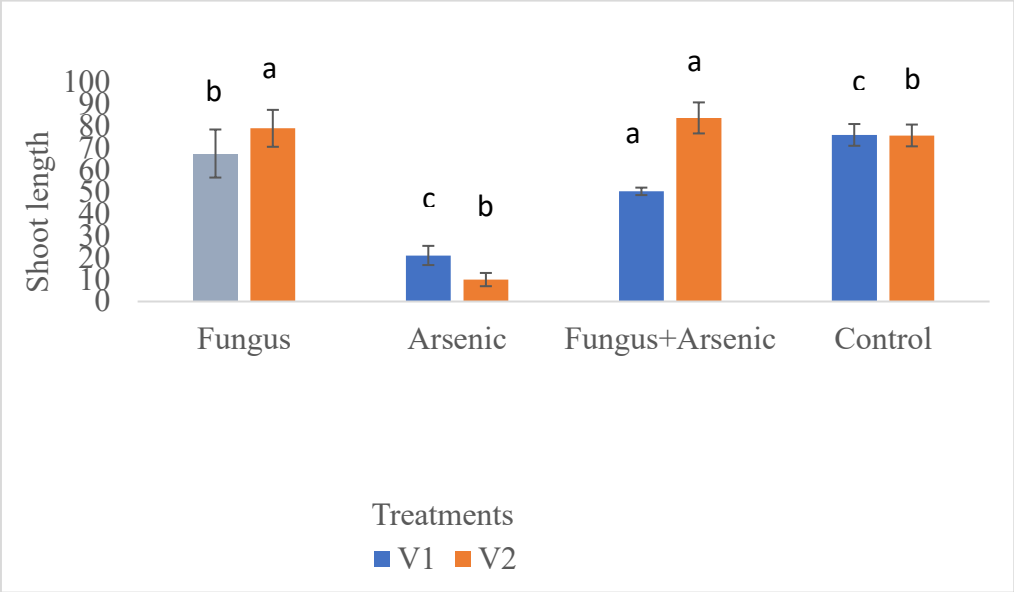

Figure 6: Shoot length of wheat grown in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

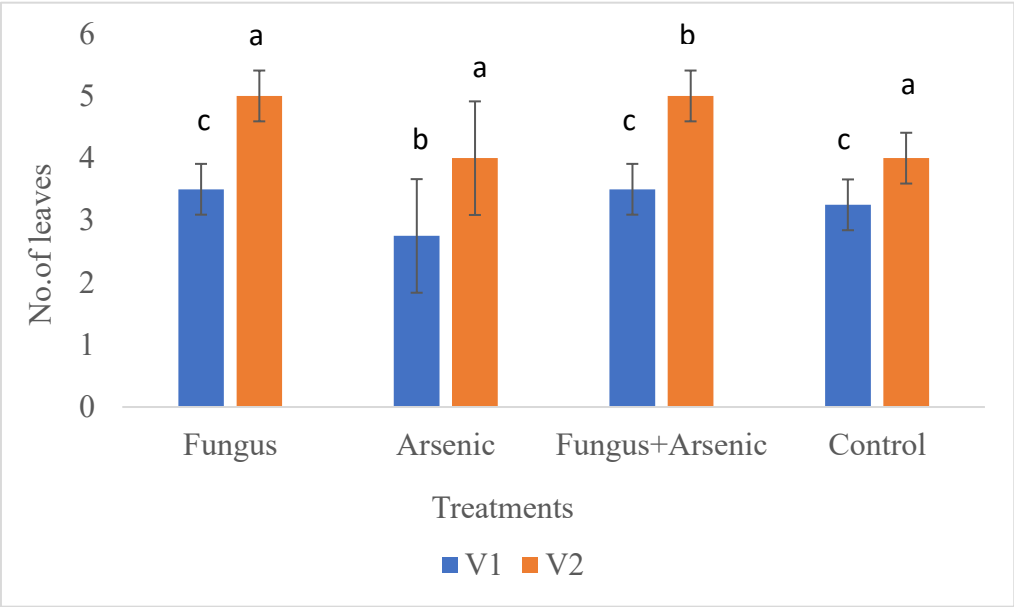

Figure 7: Number of leaves of wheat grown in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

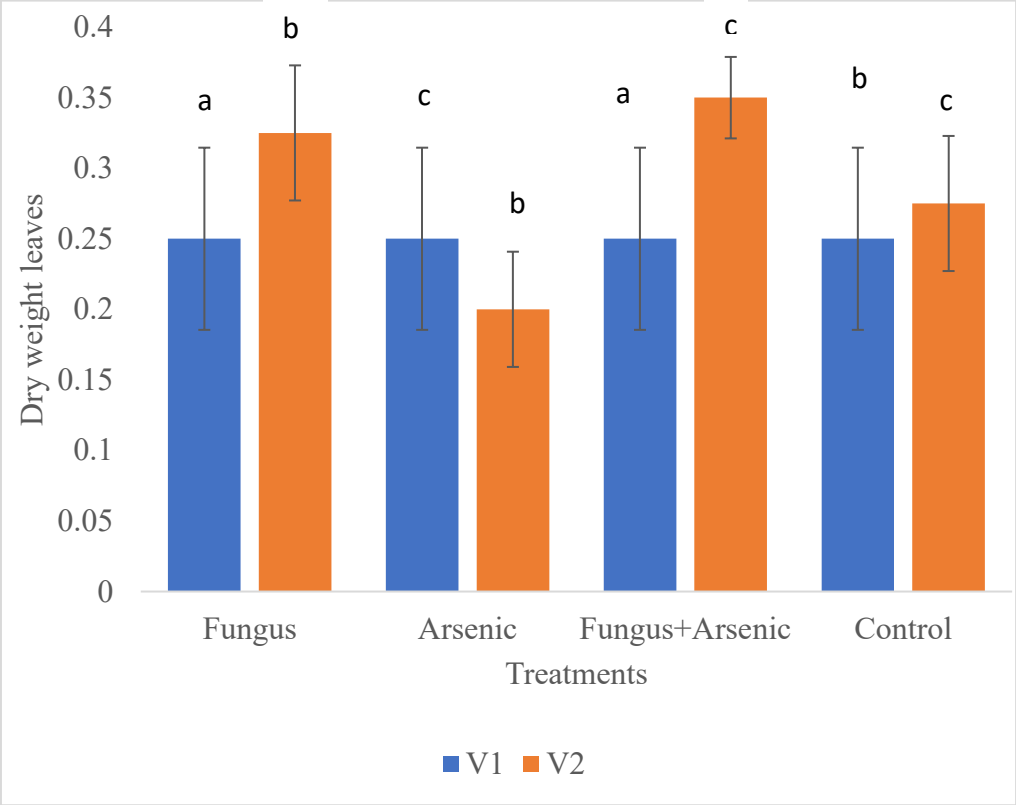

Figure 8: Dry weight of leaves grown in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

Root dry weight (g)

ANOVA was used for the measurement of dry weight of root (Figure 9). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Maximum root weight was shown by arsenic fungus treatment of V2.

Shoot dry weight (g)

ANOVA was used for the measurement of shoot dry of wheat plants (Figure 10). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Maximum shoot weight was shown by arsenic fungus treatment of V2.

Chlorophyll a (mg g–1 f. wt)

ANOVA was used for the estimation of chlorophyll a in wheat plants (Figure 11). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Maximum chlorophyll a concentration was shown by arsenic fungus treatment of V2.

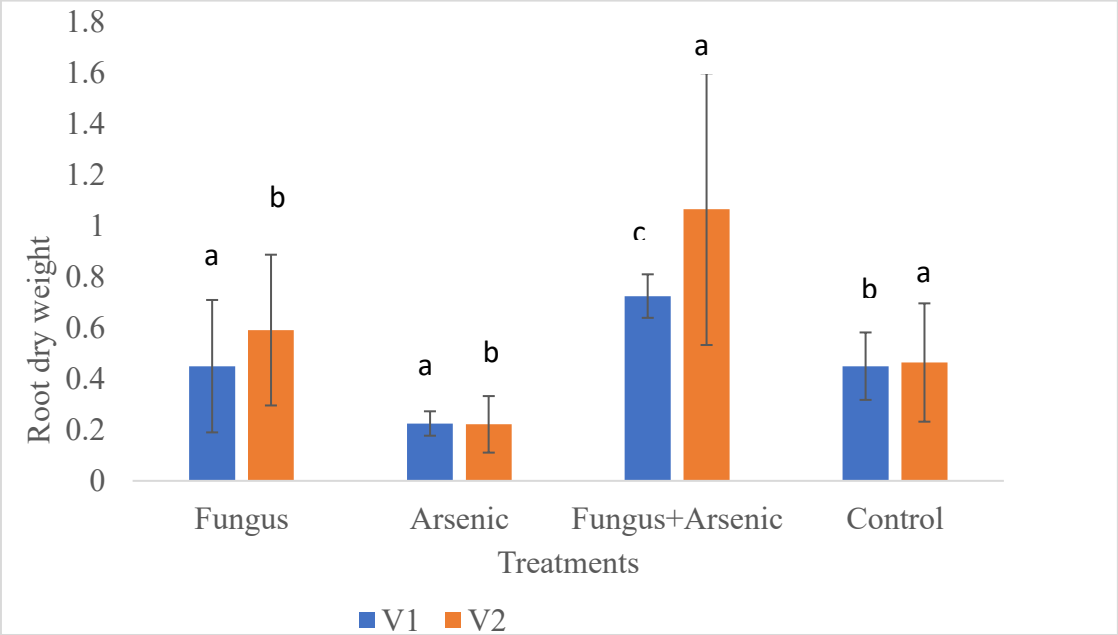

Figure 9: Root dry weight growth in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters alters above the bars, based on statistical analysis using Statistics 8.1.

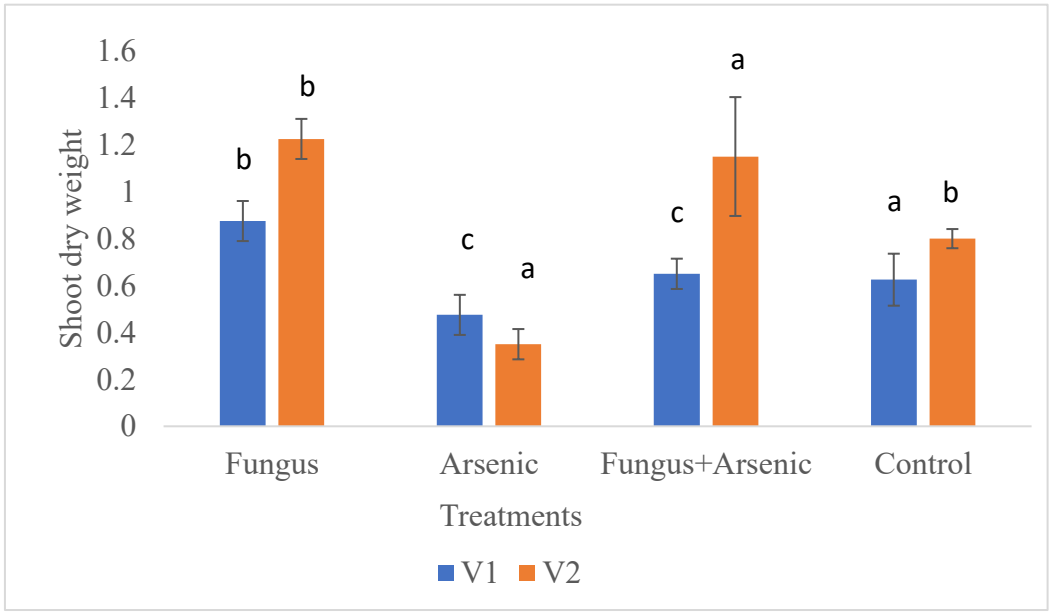

Figure 10: Shoot dry weight growth in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

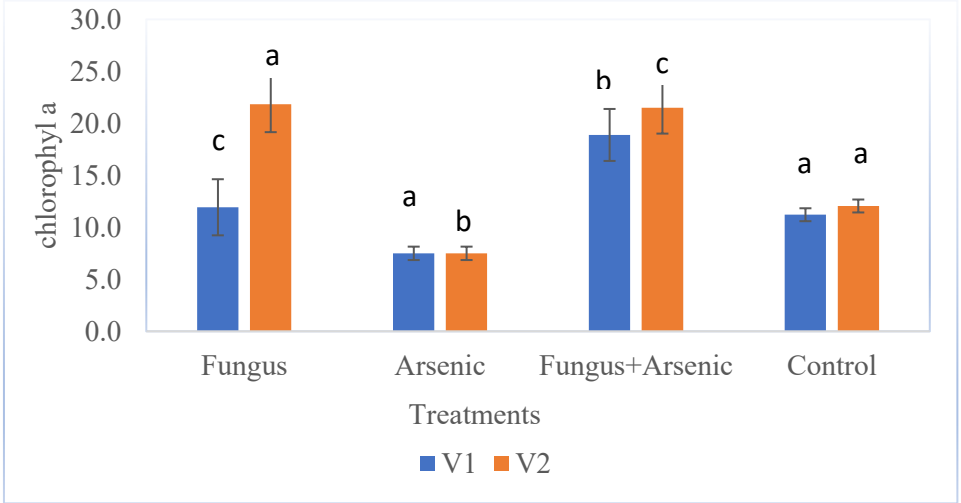

Figure 11: Chlorophyll a (mg g–1 f. wt.) concentration in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters alters above the bars, based on statistical analysis using Statistics 8.1.

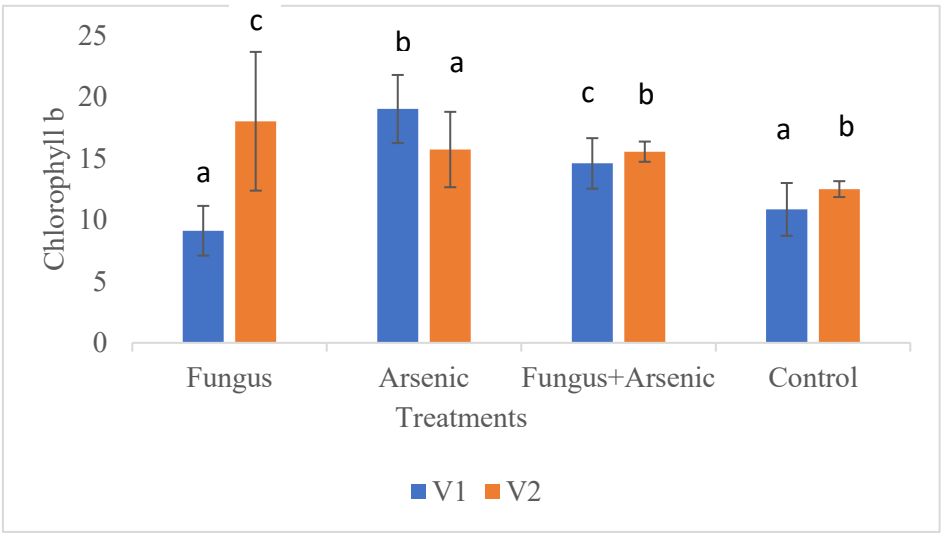

Figure 12: chlorophyll b (mg g–1 f. wt.) concentration in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters alters above the bars, based on statistical analysis using Statistics 8.1.

Chlorophyll b (mg g–1 f. wt)

ANOVA was used for the concentration of chlorophyll b in wheat plants (Figure 12). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Maximum chlorophyll b concentration was shown by arsenic fungus treatment of V2.

Total chlorophyll

ANOVA was used for the estimation of total chlorophyll (Figure 13). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. Concentration of total chlorophyll shown by arsenic fungus treatment of V2.

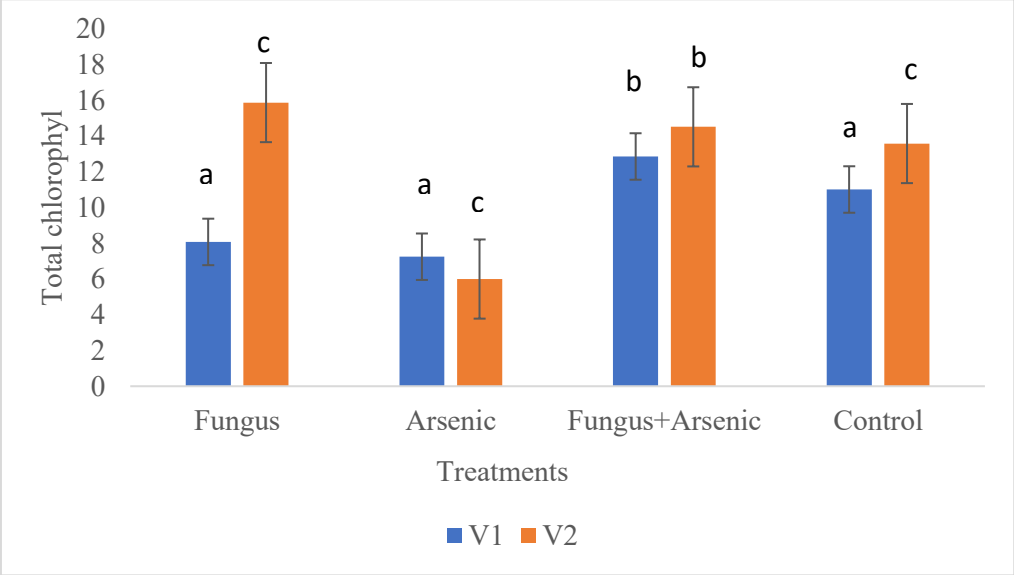

Figure 13: Total chlorophyll concentration with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

Carotenoids (mg g–1 f. wt)

ANOVA was used for the estimation of carotenoids (Figure 14). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. The highest carotenoid concentration shown by arsenic fungus treatment of V2.

Arsenic concentration in wheat

ANOVA was used for the estimation of arsenic in wheat roots, straw and grains (Figure 15). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. The highest growth of wheat in straw was shown by arsenic fungus treatment of V2.

Arsenic + fungus concentration

ANOVA was used for the estimation of arsenic + fungus inoculation (Figure 15). The fungus + arsenic factor turned out to a significant source of variation according to the main effect. Additionally, interactive effects turned out to be important contain information about a comparison of plant shoot length means. The highest growth of wheat in straw was shown by arsenic fungus treatment of V2.

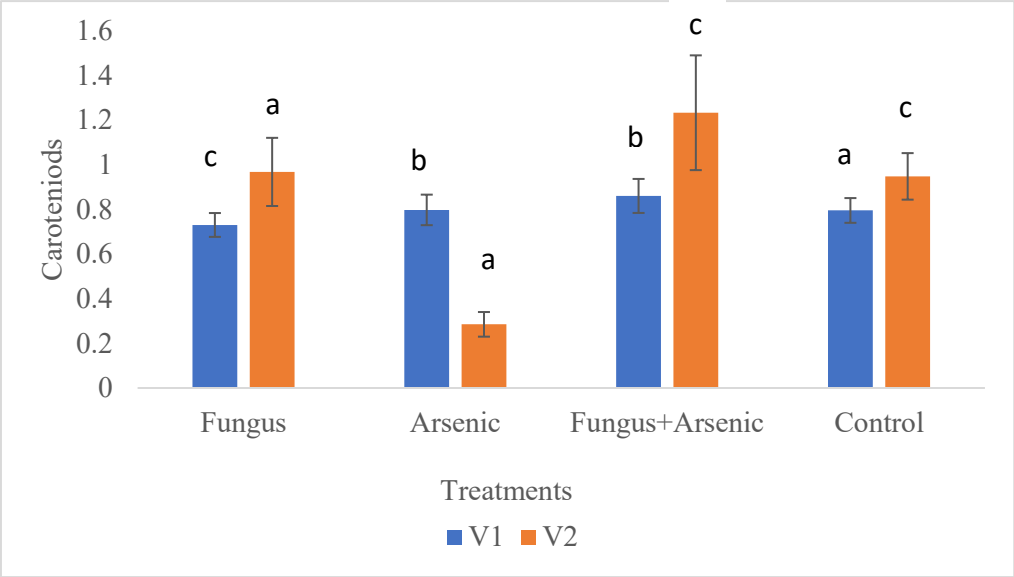

Figure 14: Carotenoids concentration (mg g–1 f. wt) with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

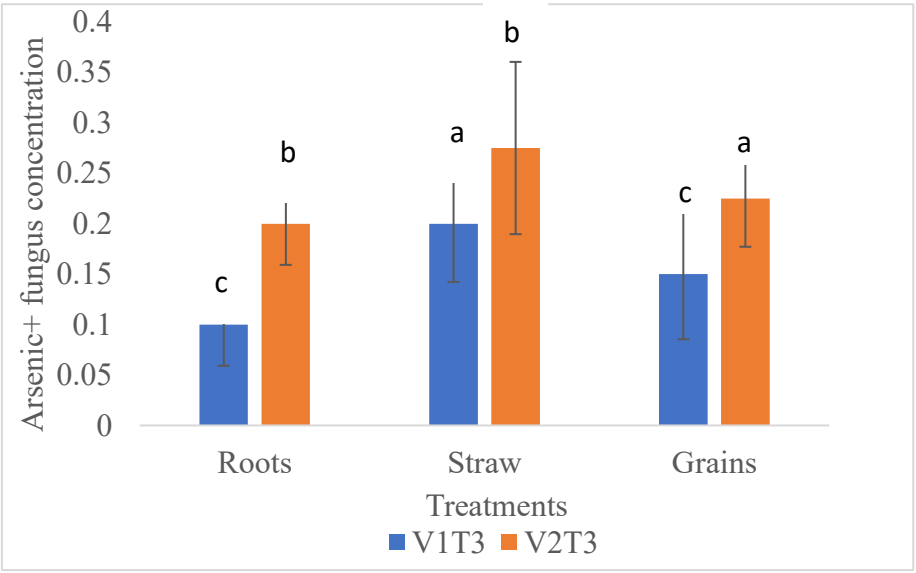

Figure 15: Arsenic + Entophytic fungus concentration in wheat growth in soil with inoculation of endophytic fungus and arsenic stress. V1 = Dilkash, V2 = Fakhr-e-Bakhar. Vertical bars show the standard error derived from four replicates. Significant differences are shown by different letters above the bars, based on statistical analysis using Statistics 8.1.

DISCUSSIONS

Results of present study showed that the inoculation of *Fusarium oxyporium* efficiently reduce Arsenic concentration from wheat plants. Utilization of *F. oxyporium* showed the noticeably reduced Arsenic concentration in rhizospheric area of *Triticum astevium* subsequently and then in shoot, leave and grain area in both cultivars i.e. V1 and V2.

The mycelial growth plays vital role in absorbing of metalic arsenic species, therefore controlling their availability and mobility in crops. Cell membrane and cell wall in fungus specie comprises different functional-groups which provides standard binding-sites in arsenics contributing, hence reduces its phyto-availability as well as mobility. Varity V2 (Fakhr-e-bakhar) showed the better growth on fungus *Fusarium oxyporium* inoculation and alleviated the arsenic stress as compared to V1 (Dilkash). In the case of Endophytic Fungi like *Fusarium oxyporium,* the conidia and mycelial growth formation starts from the rhizospheric area of the neighbouring plant which gives the wide surface area, where Asenic species sticks to them as a result decreases its harmful effect. Hence, outcome of present studies approved that *Fusarium oxyporium* showed a great impact in mitigation and in the bio-accumulation of As in different crops cultivation techniques respectively. This has noteworthy effects for the confirmation of restoring ecosystem practices in natural environments and in food security.

REFRENCES

Abaya, A., Xue, A. and Hsiang, T. 2021. Selection and screening of fungal endophytes against wheat pathogens. Biological Control, 154: 104511.

Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Niazi, N. K., Khan, M. I. and Natasha. 2018. Arsenic uptake, toxicity, detoxification, and speciation in plants: physiological, biochemical, and molecular aspects. International journal of environmental research and public health, 15(1): 59.

Abdalla, S. A., Algam, S. A., Ibrahim, E. A. and Naim, A. M. E. (2014). In vitro screening of Bacillus isolates for biological control of early blight disease of tomato in shambat soil. World Journal of Agricultural Research, 2(2): 47-50.

Afzal, M., Khan, Q. M. and Sessitsch, A. 2014. Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. Chemosphere, 117: 232-242.

Babu, A. N., Jogaiah, S., Ito, S. I., Nagaraj, A. K. and Tran, L. S. P. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. Plant Science, 231; 62-73.

Deng, Z. and Cao, L. 2017. Fungal endophytes and their interactions with plants in phytoremediation: a review. Chemosphere, 168: 1100-1106.

El-Shahir, A. A., El-Tayeh, N. A., Ali, O. M., Abdel Latef, A. A. H. and Loutfy, N. 2021. The Effect of endophytic Talaromyces pinophilus on growth, absorption and accumulation of heavy metals of Triticum aestivum grown on sandy soil amended by sewage sludge. Plants, 10(12): 2659.

Fisher, N. L., Burgess, L. W., Toussoun, T. A. and Nelson, P. E. 1982. Carnation leaves as a substrate and for preserving cultures of Fusarium species. Phytopathology, 72(1): 151-153.

Malik, A., Butt, T. A., Naqvi, S. T. A., Yousaf, S., Qureshi, M. K., Zafar, M. I. and Iqbal, M. 2020. Lead tolerant endophyte Trametes

hirsuta improved the growth and lead accumulation in the vegetative parts of Triticum aestivum L. Heliyon, 6(7).

Mandal, B.K., Suzuki, K.T. Arsenic round the world: A review. Talanta 2002, 58: 201–235.

Mandal, R., Samstein, R. M., Lee, K. W., Havel, J. J., Wang, H., Krishna, C. and Chan, T. A. (2019). Genetic diversity of tumors with mismatch repair deficiency influences anti–PD-1 immunotherapy response. Science, 364(6439): 485-491.

Nasif, S. O., Siddique, A. B., Siddique, A. B., Islam, M. M., Hassan, O., Deepo, D. M., and Hossain, A. (2023). Prospects of endophytic fungi as a natural resource for the sustainability of crop production in the modern era of changing climate. Symbiosis, 89(1): 1-25.

Niazi, N. K., Bibi, I., Shahid, M., Ok, Y. S., Burton, E. D., Wang, H. and Luttge, A. 2018. Arsenic removal by perilla leaf biochar in aqueous solutions and groundwater: an integrated spectroscopic and microscopic examination. Environmental Pollution, 232: 31-41.

Ohm, H. S., Sahadewa, A., Hryciw, R. D., Zekkos, D. and Brant, N. 2013. Sustainable soil particle size characterization through image analysis. Geotechnical and Geological Engineering, 31: 1647-1652.

Priyadharsini, P., and Muthukumar, T. 2017. The root endophytic fungus Curvularia geniculata from Parthenium hysterophorus roots improves plant growth through phosphate solubilization and phytohormone production. Fungal Ecology, 27: 69-77.

Riseh, R. S., Vazvani, M. G., Hajabdollahi, N. and Thakur, V. K. 2023. Bioremediation of heavy metals by Rhizobacteria. Applied Biochemistry and Biotechnology, 195(8): 4689-4711.

Rosas-Castor, J. M., Guzmán-Mar, J. L., Hernández-Ramírez, A., Garza-González, M. T. and Hinojosa-Reyes, L. 2014. Arsenic accumulation in maize crop (Zea mays): a review. Science of the Total Environment, 488: 176-187.

Sharma, V., Thakur, M. and Chauhan, A. 2024. Isolation, identification, pathogenicity test and molecular characterization of Fusarium oxysporum f. sp. zingiberi causing Fusarium yellows disease from infected rhizomes of ginger (Zingiber officinale Rosc.). Journal of Plant Pathology, 98: 1-6.

Smedley, P. L. and Kinniburgh, D. G. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Applied geochemistry, 17(5): 517-568.

Soto, J., Ortiz, J., Herrera, H., Fuentes, A., Almonacid, L., Charles, T. C., and Arriagada, C. 2019. Enhanced arsenic tolerance in Triticum aestivum inoculated with arsenic-resistant and plant growth promoter microorganisms from a heavy metal-polluted soil. Microorganisms, 7(9): 348.

Suwandi, Akino, S., & Kondo, N. (2012). Common spear rot of oil palm in Indonesia. Plant Disease, 96(4): 537-543.

Tipping, E., Rieuwerts, J., Pan, G., Ashmore, M. R., Lofts, S., Hill, M. T. R. and Thornton, I. (2003). The solid–solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. Environmental pollution, 125(2): 213-225.

Zheng, M., Shi, J., Shi, J., Wang, Q., and Li, Y. 2013. Antimicrobial effects of volatiles produced by two antagonistic Bacillus strains on the anthracnose pathogen in postharvest mangos. Biological Control, 65(2): 200-206.