Inoculation with *Glomus mosseae*: an efficient biological management strategy for Arsenic mitigation in wheat (Triticum aestivum L.) under Arsenic contaminated soil

MAHENDRA SINGH*, DEBJIT CHAKRABORTY, JAJATI MANDAL, DINESH KUMAR CHAUDHARY AND ARUN KUMAR JHA

Department of Soil Science & Agricultural Chemistry, Bihar Agricultural University, Sabour-813210, Bhagalpur, INDIA

***Corresponding author email address:** m.singh30648@gmail.com (Mahendra Singh)

ABSTRACT

A pot experiment was conducted to evaluate the effects of Arbuscular Mycorrhizal (AM) fungi on mitigation of arsenic (As) in As-contaminated soils with wheat (variety- HD 2967) as the test crop. Soil in bulk was collected from Bihar Agricultural University, Sabour, Bihar, India farm with physicochemical properties to conduct a pot experiment using three doses of As (5, 10, 20 mg L^{-1}) and application of *Glomus mossaea* inoculation (no inoculated, 5 g/pot and 10 g/pot). Result indicated that the treatment spiked with As 5 ppm and *Glomus mossaea* @1g kg^{-1} showed the lowest available As (21.80 µg kg^{-1} soil) and lowest total As (2.533 mg kg^{-1} soil) as compared to all other treatments. The As content in different parts of plants are found in the order of roots > shoots> leaves > grain parts in wheat crop. Arsenic uptake in grain was found to be positively correlated with available ($r=0.883$, $p<0.001$) and total As content in soil ($r=0.869$, $p<0.001$) as well. The application of AM fungi@10.0g kg^{-1} soil significantly ($p<0.005$) reduced the As content in grain under all the applied treatments. Arsenic content in grain was found to be negatively correlated with total glomalin content of soil ($r=-0.430$, $p<0.005$), colonization by AM fungi ($r=-0.261$) and available phosphorus (P) content of soil ($r=-0.864$, $p<0.001$). The study noticeably indicated that the mycorrhizal inoculation can reduce the arsenic content in the various parts of the wheat in As contaminated soils.

KEYWORDS: Arsenic, Glomalin, *Glomus*, mycorrhiza, wheat,

INTRODUCTION

Arsenic (As) contamination; a brief overview

Arsenic (As) being a trace toxic metalloid holds great environmental concern owing to its presence in soil, water, plant, animal and human continuum. Arsenic paves its entry into the soil plant system either naturally through weathering of As-bearing rocks and minerals, use of As-contaminated groundwater, or else through various anthropogenic activities such as application of As-containing pesticides in agriculture, combustion of coal, mining and smelting of base metal ores, etc. More than 20 nations from all over the world have documented groundwater arsenic poisoning and human suffering as a result, including Argentina, Chile, Finland, Hungary, Mexico, Nepal, Taiwan, Bangladesh, India, and others, to name a few (Sanyal et al., 2015). Although drinking water is the most common route of arsenic exposure, other pathways such as soil-crop-food transfer can also cause arsenic toxicity. The recommended provisional guideline value of total arsenic concentration in drinking water by World Health Organization (WHO) is 10 mg As. L^{-1}. Total arsenic content ranging from 10 to 20 ppm has found to be an index of arsenic hazard (Rahaman et al., 2013).

As in wheat: worldwide a growing concern

Presence of As in wheat and maize in the Indo-Gangetic Plain of India has been reported (Mandal et al., 2019b). Consumption of wheat is next to rice in India but has greater annual global consumption than rice. Worldwide, the annual consumption of wheat (730.9 million tonnes) is more than that of rice (506.5 million tonnes) (Food and Agriculture Organization, 2020). Although, unlike rice, wheat is not a great As accumulator, recently it is demonstrated as a significant source of As exposure to humans (Rasheed et al., 2018). A recent study suggested that wheat poses higher risk as an exposure source of As than rice precisely due to 50% higher consumption of wheat products to that of rice (Food and Agriculture Organization, 2020). Similarly, in a study from rural Bihar, India documented that concentrations of As in wheat flour was found to be high enough to cause excess lifetime cancer risk indicating widespread arsenic exposure from wheat intake in the studied population (Suman et al., 2020). Arsenate (As^{+5}) in aerobic soil conditions is the predominant As species. Arsenate competes with inorganic phosphate (Pi) since they both are transported through a similar transporter system. In an As-contaminated soil, under aerobic conditions which is ideal for wheat cultivation, the predominance of As^{+5} interferes with Pi uptake by plant, minimizes Pi uptake, thus hampers overall metabolic activity of the plant. Alike other heavy metals like cadmium (Cd), Chromium (Cr), lead (Pb), As also hampers the uptake and translocation of other essential nutrients like Mg, Zn, Fe, Mn etc, creating osmotic imbalance in the system, disruption of enzymatic activity and cause overall impairment in metabolic activity of the grown plants. It is a major concern that As toxicity together with As-induced mineral deficiency can aggravate health risks in people consuming wheat based diet, especially in a scenario where about 33% of the global population suffers from anemia, Zn deficiency, and poor mineral status (World Health Organization (WHO), 2015; International Maize and Wheat Improvement Center (CIMMYT), 2017).

Arbuscular Mycorrhizal Fungi (AMF) inoculation; a potent biological management strategy to alleviate As contamination in plant system

Therefore, As mitigation strategy is necessary for wheat cultivation. The only possibility of reducing human exposure to this hazardous metalloid is to restrict its uptake and subsequent translocation to wheat grain while maintaining its essential nutritive value. Biological management strategies to alleviate heavy metal toxicity is gaining significant importance keeping in view the conception of sustainable development goals (SDGs) running currently by United Nations Organizations (Food and Agriculture Organization, 2020). Arbuscular mycorrhizal fungi (AMF) mostly belonging to the division glomeromycota of order glomerales is one of the significant fungal species inhabiting in the root cortex of almost all agricultural crop species. Earlier studies have reported that colonization by arbuscular mycorrhizal fungi (AMF) improved growth and yield of wheat plants under As stress (Sharma et al., 2017; Gupta et al., 2021). Arsenite (As^{3+}) triggers the generation of reactive oxygen species (ROS) that worsens the oxidative stress in a plant resulting in up regulation of oxidative biomarkers like hydrogen peroxide (H_2O_2), malondialdehyde (MDA) etc. Those oxidative biomarkers having been up regulated damage the plasma membrane causing lipid peroxidation, peroxidation of proteins and ultimately result in overall cell damage leading to necrosis. The inoculation of *Rhizophagus irregularis* in an experiment conducted by Albqmi et al., (2023) had improved antioxidant enzymes activity like superoxide dismutase (SOD), catalase

(CAT), peroxidase (POX), glutathione reductase (GR), and glutathione peroxidase (GPX) who enacted as a trap for H_2O_2 by scavenging it and ultimately converting it into molecular oxygen (O_2) species. By virtue of this MDA levels also got reduced. Additionally, the inoculation of AMF boosts plants to produce polyphenolic compounds like flavonoids, tannins, phenolic acids who also being potential reducing agents alleviate the toxic effect of ROS and MDA as well by scavenging them and converting into less reactive, less toxic molecular species. This is how AMF inoculation helps plant tolerate and alleviate As stress. This statement is well supported by the conclusions generated by Mondal et al., (2022). The role of AMF in biofortification of essential minerals, especially Cu, Fe, Ni, Se, and Zn is reported in cereal crops (Goicoechea et al., 2016; Coccina et al., 2019). *Glomus mossaea* is a significant AMF in the context of inhabiting in the root cortex of almost 90 % of agricultural crop species.

Inoculation with *Glomus mossaea*; prospects

Glomus mossaea is considered as one of the best biological management strategy for enhancing plant growth and shoot biomass as it can detoxify As induced stress. *Glomus mossaea* reduces As stress by As immobilization in fungal structure, precipitation and chelation in the rhizosphere, sequestration in vacuoles and activation of antioxidant mechanisms in plants. Generally As gets immobilized in the fungal hyphae living in symbiotic association with plants, which reduce their availability to plants by retaining the As in the cell wall, vacuole or cytoplasm by chelation, thus decreasing metal toxicity in the plants. The fungal cell wall has specific metal-binding peptides, proteins, and polysaccharides that contain hydroxyl (HO^-), carboxyl (R-COOH), phosphate (PO_3^-), sulfate (SO_2^{4-}), and amino (NH_2) groups that bind metalloid ions to restrict their phytoavailability (Kumar et al., 2021 and Hassan et al., 2020). Several different strategies have been ratified for how *Glomus mossaea* influence the uptake, translocation and accumulation of Heavy Metals (HMs) and enhance plant tolerance to HMs stress. These strategies include various steps viz. the retention of HMs in the mycorrhizal roots and external hyphae, the stimulation of nutrient absorption, the sequestration of HMs in vacuoles, the binding of HMs on the fungal cell wall, the protection of the reaction center and the rectification of gas exchange capacity, the increasing of the antioxidant response of plants, the chelation of HMs in the cytosol of fungi and the induction of glomalin by AMF, and AMF-mediated Phytoremediation. Glomalins are the glycoproteins produced by AMF and exist as homologs of heat shock protein 60 (hsp 60) that play a role in the immobilization of HMs. In an experiment conducted by Anupama and Chamola (2023), it was revealed that inoculation of *Glomus macrocarpum* and *Glomus fasciculatum* as bioinoculants in *Leuceana leucocephala* plant had ensured significant increase in plant growth, biomass, chlorophyll and carotenoids content when grown under As stressed soil. Likewise although there are several reports of *Glomus mossaea* being successful in reducing the uptake of As and heavy metals in different crops, studies in wheat are scanty. So, in this present study, a modest initiative has been undertaken to explore the efficacy of *Glomus mossaea* in diminishing As uptake in wheat grain which can serve as a potential mitigation strategy for the wheat growing As endemic areas of Bihar, India.

MATERIALS AND METHOD

Pot experiment

A pot experiment was conducted in the Department of Soil Science and Agricultural Chemistry, Bihar Agricultural University, Sabour, Bhagalpur, Bihar (87º2’42”East and latitude of 25º15′40″ North) during rabi season’2019. The soil in bulk was collected from Bihar Agricultural University, Research farm belongs to Inceptisols which is well drained and silty loam in texture. Total twenty-seven (27) plastic pots were filled with 10 kg of soil and all the pots were spike with arsenic dose as per the treatment: 0ppm, 5.0 ppm, 10.0 ppm and 20.0 ppm, after application of As the *Glomus mossaea* inoculum (*Glomus mossaea* was commercial products of The Energy Resource Institute

(TERI), New Delhi, India) @ 0 g, 5.0g and 10.0/pot (IP-20 g^{-1}) were applied 2-3 cm beneath the wheat seed (Variety-HD2967) seed on 16th November, 2019. The treatments (T) were set up in nine (9) different treatments likely, T1-As 05ppm + *Glomus mossaea* @0 g/pot, T2-As05 ppm+ *Glomus mossaea* @5.0g/pot, T3-As05ppm+ *Glomus mossaea* @10.0 g/pot, T4-As 10 ppm + *Glomus mossaea* @0 g/pot, T5-As 10 ppm+ *Glomus mossaea* @5.0 g/pot, T6-As 10 ppm+ *Glomus mossaea* @10.0 g/pot, T7-As20ppm + *Glomus mossaea* @0 g/pot, T8-As20 ppm+ *Glomus mossaea* @5.0g/pot and T9-As 20 ppm+ *Glomus mossaea* @10.0 g/pot. The soils in the pots were treated with recommended doses of N: P: K (100:40:20 Kg ha^{-1}) using reagent grade Urea and Potassium di-Hydrogen Phosphate irrespective of the treatments. The total P and K fertilizer were applied at basal dose while N fertilizer were applied in three splits doses 50% at basal and rest 50% top dressed at crown root initiation (CRI) and at booting stage. Altogether 5 wheat plants were maintained in the pots. The pots were irrigated 5 times throughout the crop growth period with distilled water. The experiments were laid out as Completely Randomized Design (CRD) with three replications. Wheat grain was harvested at full maturity on 17^{th} April, 2020.

AM colonization

The root samples with adhering soil were collected at 50 days after sowing (DAS). They were washed repeatedly with sterilized distilled water and fragmented into small segments of 1 cm. The root segments were cleared in 10% KOH and stained with 0.5% Trypane blue by the method given by Phillips and Hayman (1970). The stained bits were examined and the Arbuscular mycorrhizal colonization in the roots was recorded in terms of per cent root segments showing mycorrhiza formation.

Glomalin quantification:

Total Glomalin (TG) extraction was done by using autoclaving using 50 mM sodium citrate, pH 8.0. One to two g of soil was taken in a centrifuge tube with 8 ml 50 mM sodium citrate for total Glomalin extraction Glomalin (Rillig, 2004; Rosier et al., 2007; and Rillig, 2003; Wright et al., 1996; Wright and Jawson, 2001; Wright, Nichols, & Schmidt, 2006; Wright & Upadhyaya, 1996; & Wright & Upadhyaya, 1998). The cap tubes were put on vortex to have appropriate soil: solution contact. Autoclave for 90 min at 121^{o} C for 60 minutes, after autoclaving it was centrifuge at 10000 xg for 10 min immediately after extraction. The centrifugation was done just to pellet the soil particles. Remove the supernatant containing the protein and store at $4^{o}C$. The autoclaving and centrifugation was repeated five times to get straw colour. One ml supernatant was transfer into the microtitre tube and protein was quantify with Bradford Protein Assay (He, F. 2011).

Soil sampling and analysis of soil and plant samples

The rhizosphere soil collected from each pot was air dried, grinded and sieved with 2 mm sieve. They were analyzed for pH (1:2.5 soil-to-water ratio), clay content (International pipette method), organic carbon (OC) (Walkley and Black, 1934), available Nitrogen content of soil was determined by the Kjeldahl method (Subbiah and Asija, 1956), available Phosphorus using 0.5 mol L^{-1} NaHCO3 (pH 8.5) (Olsen et al., 1954), exchangeable Potassium using 1 mol L^{-1} NH4OAc (pH 8.80) (Jackson, 1973). Tri-acid, HNO_3, $HClO_4$ and H_2SO_4, in a proportion of 10:4:1 (v/v/v) was used for total As analysis in plant samples (Mandal et al., 2019). Available As in soil was determined using 0.5 mol L^{-1} NaHCO3 (pH 8.5) (Sparks, 2003) and Total As in soil sample was determined by using the sample which was digested in tri-acid mixture of trace element grade reagents (HNO_3:H_2SO_4:$HClO_4$::10:1:4, v/v) outlined by (Johnston and Barnard, 1979). The pH of the collected soil sample was 7.42. The organic carbon (OC) percentage was observed to be 0.57 in the soil sample and belonged to silty clay loam. The available N was found to be 255.14 kg ha^{-1} and it was found deficient in available N. The available P was 10.25 kg ha^{-1} and available K was 183.42 kg ha^{-1}. The available and total As were 0.36 mg kg^{-1} and 4.06 mg kg^{-1} in the used soil respectively.

Instrumentation and analytical conditions for As analysis

The extract from soil or the digest of soil and plant samples were prepared as per the methods outlined by (Mandal et al., 2019a). The resultant solution was analyzed in an Agilent 240 FS atomic absorption spectrophotometer with Vapor generation Accessory (VGA 77). In every batch of 30 samples two reagents blank and one standard reference materials of wheat (SRM1568a), prepared by National Institute of Standards and Technology (NIST) were used. The certified value of SRM1568a is 290 ± 30 mg kg^{-1}. Analysis of standard material was carried in triplicate and As content was recorded as 285 ± 10.1 mg kg^{-1}.

Statistical analysis

All the data obtained from the experiments was analyzed using Statistical Package for the Social Sciences 20.0 (IBM SPSS 20). One-way ANOVA was performed for comparing significant differences among individual means and Bonferroni multiple comparison analysis done for comparison among treatments. The statistical analysis conducted in this experiment was followed by the standard protocol as IBM Corp. (2020).

RESULTS AND DISCUSSION

As uptake by grain

Arsenic concentrations in roots, leaves, and grains elevated proportionally to the level of As amendments in soil. Results of the study showed that at 20 ppm As, grains of non mycorrhizal plants accumulated 0.26 mg kg^{-1} DW of As (Fig. 1), which is higher than the WHO recommended permissible limit (1.0 mg kg^{-1}) of As in cereal grains (Bhattacharya et al., 2010). Kundu et al. (2013) also reported that As concentration in wheat could exceed the permissible limit of As when grown in soil contaminated with elevated levels of As. This indicates that wheat represents a risk factor for human health when grown in As contaminated soil. However, in agreement with earlier studies (Sharma et al., 2017; Gupta et al., 2021), colonization by *R. intraradices* decreased the accumulation of As in plant parts. Even at 50 ppm As level, concentration of As in grains of M-plants (0.5 mg As kg^{-1} DW) was below the WHO recommended permissible limit of As.

It has been observed from the experimental data that with increasing the level of As in soil the As uptake (Figure 1) was found to increase significantly (p=0.05). While the minimum (69.42 µg pot^{-1} and 99.94 µg pot^{-1}) and maximum (79.05 µg pot^{-1} and 115.45 µg pot^{-1}) increment in As uptake by grain was recorded in the soil spiked with As 5 ppm and As 20 pm respectively in non mycorrhizal plants. The inoculation of *Glomus mosseae* significantly decreased the As uptake by grain when compared with non inoculation with respect to all levels of As contamination soil. The inoculation of *Glomus mosseae* @ 10.0 g/pot along with As 05 ppm treated pot (T3) had registered lowest As content in the grain of the grown wheat crop under that treatment significantly than non mycorrhizal high As (20 ppm) treated pot. Also, the inoculation of *Glomus mosseae* @ 10.0 g/pot along with As 05 ppm treated pot (T3) had given best result under this regard. The inoculation of the AM fungi there had lowered down the available As (2.53 mg kg^{-1}) as well as total As (21.80 mg kg $^{-}$1) content of soil. It might be due to the immobilization of mobile, available As within the fungal mycelia and sequestration by the glycoprotein, glomalin secreted by the AM fungi and thus reducing phytoavailability. Chelation or sequestration of As in fungal vacuole, binding with the chitinous fungal cell wall is also documented in several studies. These sorts of things could attribute in lowering down of available as well as total As content in soil, simultaneously reduced As uptake and/or content in the grain portion. Similar kind of finding was also reported by Garg and Cheema (2021) where in *Rhizoglomus intraradices* inoculated *Cicer arietinum* (chickpea) plant, carbohydrate metabolism was up regulated, biomass production was maximized and in the rhizosphere region of that crop excretion of glomalin related soil protein (GRSP) was found to be way superior to rhizosphere of non-inoculated plants. This GRSP being a glue like glycoprotein in its broken edge contain 1-9 percent iron (Fe^{+3}), which has high affinity towards arsenate As(V) species of As, by this As gets sequestered inside the structure of GRSP rendering its phytoavailability thus diminished uptake by the crop is well ensured as well. Very similar kinds of findings are documented by Anli et al., (2022) as well. Also, the inoculation of *Glomus mosseae* @ 10.0 g/pot along with As 05 ppm treated pot had registered highest available phosphorus (P) content in soil, thus maintaining the highest P:As ratio among all the other treated pots. Phosphorus and As due to having been transported by similar transporter systems are antagonistic inside the plant system. The highest available P thus might result in lowest uptake and/or content of As by the grains for that respective treatment. The study by Orlowska et al., (2012) documented that colonization by AM fungi could reduce the As uptake in roots, shoots and grains of lentil crops.

Root colonization, total Glomalin and As content in wheat plant parts

Root colonization

The root cortex of mycorrhizal plants showed significantly more presence of arbuscules, vesicles and hyphae (Table 1) over non mycorrhizal plants at all As levels. Furthermore, the soil inoculation with *Glomus mossaea* @10.0 g/pot$^-$+As (5.0 ppm) gave significantly higher root colonization when compared with treatments As (5.0 ppm) without *Glomus mossaea* , *Glomus mossaea* @5.0 g/ pot +As (5.0 ppm), As (10.0 ppm) without *Glomus mossaea* , *Glomus mossaea* @5.0 g/ pot$^-$+As (10.0 ppm), *Glomus mossaea* @10.0 g/ pot +As (10.0 ppm) and As (20.0 ppm) without *Glomus mossaea* inoculation. Higher mycorrhizal infection (%) was observed in root and rhizosphere soil of mycorrhizal plants. With increasing arsenic concentration in soils (from 5 ppm to 20 ppm), fungal activity in the rhizosphere region was considerably reduced, which stopped spreading of fungal hyphae in plant root systems, thus lowering the infection rate. The functional groups of microbes which are present on the cell wall and cell membrane had bound with As, thereby binding with proteins, PO_3^-, and HO^- groups of nucleic acids such as DNA/RNA. This led to impairment of nucleic acid synthesis, functions of nucleic acids, disruption in the overall process of central dogma and eventually caused the proteins to denature, thereby inhibiting the cell division which is the most substantial part of microbial growth (Naseem et al., 2021 and Gupta et al., 2022). Similarly, other previous studies found a range of mycorrhizal responses to the presence of toxic metals (e.g. Chao and Wang 1991; Vidal et al. 1996). Ahmed et al. (2006) reported a significant reduction and complete inhibition of AM colonization in the root of seedlings planted in metal-polluted soils. The experimental results were also in agreement of the results of Ahmed et al. (2006) who found that arsenic addition above 1 mg L^{-1} significantly ($p < 0.001$) reduced the mycorrhizal infection percentage in plant roots. At the highest level (10 mg L^{-1}) of arsenic addition, they observed only 6% of the root length was infected by mycorrhiza.

Total Glomalin content

Glomalin is a glycoprotein produced abundantly in the spores and hyphae of AM fungi belonging mostly in the order of glomerales, division; glomeromycota. Glomalin along with humic acid is thought to be a significant component of soil organic matter that binds with mineral particles to aggravate soil structural stability and thus improving soil quality. The soil inoculated with *Glomus mossaea* @10.0 g/pot+As (5.0 ppm) experienced significantly higher total glomalin content when compared with treatments As (5.0 ppm) without *Glomus mossaea* , *Glomus mossaea* @5.0 g/ pot +As (5.0 ppm), As (10.0 ppm) without *Glomus mossaea* , *Glomus mossaea* @5.0 g/ pot+As (10.0 ppm), *Glomus mossaea* @10.0 g/ pot +As (10.0 ppm), *Glomus mossaea* @10.0 g/ pot +As (20.0 ppm) and As (20.0 ppm) without *Glomus mossaea* inoculation. *Glomus mossaea* at highest dose (10 g/pot) produced highest glomalin content when it was put under minimum As stress (5 ppm) over higher As (10 ppm, 20 ppm) stress. When the dosage of As went on increasing, it adversely affected nucleic acid synthesis, functioning of nucleic acids, malfunctioning as well as denaturation of existing proteins for the fungi. Overall metabolic activity and growth in terms of colonization were hampered to some extent. This reflects to the significant reduction in glomalin secretion by *Glomus* in heavy As stress when compared with low or minimum As stress. Naseem et al., 2021 also documented that, glomalin production was maximized by AM fungal species when they were put under minimum heavy metal stress.

As content in various plant parts

As a general pattern, As concentration in plant parts increases with increase in the soil. Furthermore, it has been found that the arsenic content in different plant parts followed the order root > stem > leaf > grain irrespective of all the As levels. The ranges of As content in wheat grown in soil contaminated with As5, As10 and As20 ppm were found to significantly ($p=0.05$) increase with increasing the level of As in soil without inoculation of *Glomus mossaea*. The As concentration in root, stem, leaf and grain significantly ($p=0.05$) decreased among all the levels of As with inoculation of *Glomus mossaea* inoculated plants when compared with non inoculated plants. The reduction of As levels across all inoculated treatments can be attributed to the mycorrhizal-induced production of various root exudates, which may potentially chelate with As, thereby limiting its mobility and availability to plants. This is in conformity with the findings of

AbdElgawad et al., (2022) who in an experiment conducted in wheat and soybean crop used *Rhizophagus irregularis* as bioinoculant. They have observed both the crops inoculated with *Rhizophagus* to improve sugar metabolism and both the crops had contained elevated sugar levels which led to increase the number of carbon (C) skeletons inside the plant system. Increased number of C skeletons resulted in more production of organic acids as exudates which in turn helped the plant reduce As uptake. Similarly other previous studies have also demonstrated that arbuscular mycorrhizal (AM) fungi can prevent As uptake and translocation to aboveground plant parts by selectively discriminating against it, as evidenced by a lower translocation factor (TF) in mycorrhizal plants compared to non-inoculated plants (Sharma et al., 2017; Gupta et al., 2022). *Glomus mossaea* might also reduce metal exposure of plants by uptake of metals into their structures such as the external mycelium (Kaldorf et al., 1999), the chitious cell wall (Zhou, 1999), the extra-cellular glycoprotein, glomalin (Gonzalez-Chavez et al., 2004) and fungal vesicles (Weiersbye et al., 1999). There have been suggested two possible reasons for *Glomus mossaea* mediated arsenate resistance: (1) AMF colonization might down-regulate the high-affinity phosphate/arsenate transport system and (2) AMF might increase the efflux of As (as arsenite) from mycorrhizal roots (Chen et al., 2007a). The similar results were observed by the Gupta et al., 2022. who conducted an experiment to assess the effectiveness of *Rhizophagus intraradices* on acquisition, translocation, and accumulation of minerals in grains of wheat grown in three As levels (0, 25, and 50 mg As kg^{-1} soil) and found that inoculation of *R. intraradices* decreased As accumulation, increased macronutrients uptake, and ensured higher accumulation of N, P, K, Ca, and Mg in grains. The ranges of As were likely in root (0.82-1.22 mg kg^{-1}), stem (0.12-.29 mg kg^{-1}), leaf (0.41-0.70 mg kg^{-1}) and grain (0.13-0.26 mg kg^{-1}). It was also observed that the As content in all the plant parts varied significantly with the doses of arsenic level in soil. The order of As accumulation in root > straw > leaf > grain in rice was also reported by (Das et al., 2013). The above trend of accumulation indicates increased bioaccumulation during plant growth stages whereas the translocation was reduced within the plant system. In root, the absorption is always higher. The mycorrhiza inhibiting in root cortex of the host plant, can adsorb the metalloid in arbuscule, vesicles, mycelium they have formed, also As can get sequestered into fungal vacuoles, cell wall as well. It provides a potent detoxification measure for AM fungi associated plants. This is how AM fungi induces a regulatory effect on As translocation to various aboveground portions, maintaining lower level of As in grains and leaves of inoculated plants over non-mycorrhizal plants (Zhang et al., 2021). Similar findings were also obtained by (Imamul et al., 2011). In spite of the lower As concentration under mycorrhizal conditions, the As content in all mycorrhizal plants were significantly higher than in the non-mycorrhizal. This can be explained by the higher biomass of mycorrhizal plants but also by the fact that AMF inoculated plants had more roots, so higher As uptake might be expected. Owing to the fact that As is similar to P in its physicochemical properties, arsenate [As(V)] may compete with P for sorption sites on the surface of soil particles or roots because As and P are transported across the plasma membrane via the same P transporter systems (Adriano, 2001; Jackson and Miller, 2000; Meharg and Hartley-Whitaker, 2002). The findings here are also in conformity with the findings of (Boorboori et al., 2022).

Effect on various soil properties (pH, organic carbon (OC) and As content in soil)

The Table 2 depicts the soil pH, OC, available and total As content in experimental used soil.

pH

The soil pH ranged from 7.76 to 8.54 among overall treatments decreased with inoculation of *Glomus mossaea*. A decrease in pH of soil was observed from the initial status which might be due to the rhizospheric (R:S) effect of wheat crop. The production of Carbon di-oxide (CO_2) due to bacterial respiration along with its accumulation is the main reason for decrement in soil pH for experimental soils..

Organic Carbon (OC)

The OC content in soil ranged from 0.45 to 0.55% which was statistically non significant. The *Glomus mosseae* play an important role in the secretions of glycoproteins called glomalin that led to the buildup of the organic carbon in soil. The buildup of OC in *Glomus mosseae* treated pot might be attributed to the secretions and deposition of glomalin in soil for that respective pot (Ponnamperuma et al., 1972, Zhang et al 2021).

As content in soil

The available As decreased significantly (p=0.05) from 4.31 mg kg^{-1} in As05 to 3.53 mg kg^{-1}, 9.17 mg kg^{-1} in As10 to 5.93 in As10 and 7.65 mg kg^{-1} in As20 to 12.16 in As20 by the inoculation of *Glomus mossaea* @10 g/pot. The similar trends were recorded for total As concentration in post harvested soil. Arbuscular mycorrhizal fungi (AMF) colonized with wheat roots deter As uptake and reduce As toxicity through the symbiotic relationship between each other (Li et al., 2015). The similar results obtained by the Mandal et al., 2019. Inoculation of *Glomus mossaea* @10.0 g pot $^{-1}$ along with As level of 05 ppm reduced the available as well as total As content in soil. The several detoxification measures by AM fungi might reduce the As content in the soil. The AM fungi inoculated acted as a phosphate (P) mobilizer, ensured highest available P in that pot, maintained highest P: As ratio in soil. *Glomus mossaea* are able to increase the surface area and absorption zone of mycorrhizal roots and thus improve nutrient uptake, especially P, thus improving plant growth (Wang et al., 2005). Similar results were also obtained in an experimental finding of Chen et al., 2006. The results are also in the conformity of the results of Sharma et al., 2017.

Partial Pearson Correlation between parameters

Pearson's correlation analysis was used to explore the strength of relationships among As concentration in plant parts, As uptake, Glomalin content, AM colonization and available phosphorus in soil and other soil properties. The Pearson's correlation (Table 3) revealed that As concentrations in plant parts were found to be negatively correlated with grain yield, total glomalin content, AM fungi colonization, available P in soil, soil pH and oxidizable organic carbon content in soil ($p=0.01$ and $p=0.05$), while As concentrations in different plant parts was found to be positively correlated with As uptake by grain, available As content in soil and total As content in soil, respectively ($r=0.949$, $n=27$, $p<0.001$). Similarly, available As content in soil as well as total As content in soil were negatively correlated with soil pH and organic carbon content, respectively. However, available P was found positively correlated with the grain yield ($r=0.886$, $n=24$, $p<0.001$), Glomalin content ($r=0.516$, $n=24$, $p<0.001$) and AM colonization ($r=0.389$, $n=24$, $p<0.005$) respectively. As uptake by grain was found to be negatively correlated with grain yield, Glomalin content, AM colonization and available phosphorus.

CONCLUSION

Based on this experiment, we have reached the following conclusions: the application of *Glomus mossaea* effectively reduces As concentrations in contaminated soils. The application of *Glomus mossaea* has also been shown to reduce As accumulation in different parts of plants, with the highest levels observed in the roots, followed by the shoots, leaves, and grain of the wheat crop. The development of fungal mycelium plays a crucial role in adsorbing mobile As species, thereby limiting its mobility and availability to plants. The cell wall and/or membrane of the fungal species contain various functional groups that serve as preferential binding sites for As, contributing to its reduced mobility and phytoavailability. In the case of arbuscular mycorrhizal (AM) fungi like Glomus, the arbuscules and vesicles formed in the root cortex of the host plant provide surfaces where mobile As species can adhere and ultimately be sequestered into non-mobile and unavailable forms, thus mitigating its toxic effects. The results of this experiment confirm that under the given conditions, *Glomus mossaea* can play a crucial role in mitigating the bioaccumulation of toxic metals and metalloids like arsenic in various cropping systems. This has significant implications for ensuring food safety and restoring ecosystem services in natural environments.

FUTURE SCOPE OF RESEARCH

We have finally observed from this experiment that there is a great scope to mitigating the As content in wheat grain in As contaminated soil by adopting new agronomical practices like application of *Glomus mossaea* with proper dose and organic amendment with balanced inorganic fertilizers. Considering soil as a multivariate, heterogeneous, dynamic body, this experiment can be undertaken in different climatic, soil conditions as well as under different cropping systems.

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Table 1:- Assessment of *Glomus mossaea* on As accumulation (mg kg^{-1}) in wheat plant parts

Treatment	AM colonization (%)	Total Glomalin (mg g^{-1} soil)	Root	Stem	Leaf	Grain
T1-As05ppm + *Glomus mossaea* @0 g/pot	20.00^{a}	8.361^{ab}	0.94^{d}	0.52^{d}	0.22^{bc}	0.17^{de}
T2-As05 ppm+ *Glomus mossaea* @5.0g/pot	26.00^{a}	10.276^{ab}	0.87^{e}	0.47^{d}	0.17^{d}	0.15^{ef}
T3-As05ppm+ *Glomus mossaea* @10.0 g/pot	31.33^{a}	22.602^{a}	0.82^{e}	0.41^{e}	0.12^{e}	0.13^{f}
T4-As10ppm + *Glomus mossaea* @0 g/pot	22.00^{a}	7.798^{ab}	1.07^{b}	0.62^{bc}	0.24^{bc}	0.21^{bc}
T5-As10 ppm+ *Glomus mossaea* @5.0 g/pot	27.33^{a}	17.906^{ab}	0.96^{d}	0.51^{d}	0.16^{de}	0.16^{def}
T6-As10ppm+ *Glomus mossaea* @10.0 g/pot	29.33^{a}	13.856^{ab}	0.99^{cd}	0.57^{c}	0.20^{cd}	0.19^{cd}
T7-As20ppm + *Glomus mossaea* @0 g/pot	21.00^{a}	7.312^{b}	1.22^{a}	0.70^{a}	0.29^{a}	0.26^{a}
T8-As20 ppm+ *Glomus mossaea* @5.0g/pot	24.67^{a}	9.308^{ab}	1.17^{a}	0.65^{b}	0.25^{ab}	0.23^{ab}
T9-As20ppm+ *Glomus mossaea* @10.0 g/pot	28.33^{a}	9.899^{ab}	1.05^{bc}	0.60^{bc}	0.22^{bc}	0.19^{cd}

Values followed by the same small letters do not differ significantly ($p < 0.05.$) between treatments.

Table 2: Effect of *Glomus mossaea* on soil properties in arsenic spiked soil

Treatments	**pH**	**OC (%)**	**Available As (mg kg^{-1} soil)**	**Total As (mg kg^{-1} soil)**	**Available P (mg kg^{-1} soil)**
T1-As05ppm + *Glomus mossaea* @0 g/pot	8.00^{ef}	0.46^{a}	4.31^{f}	28.66^{f}	20.52^{e}
T2-As05 ppm+ *Glomus mossaea* @5.0g/pot	8.36^{bc}	0.48^{a}	2.96^{g}	24.40^{f}	28.72^{b}
T3-As05ppm+ *Glomus mossaea* @10.0 g/pot	8.54^{a}	0.55^{a}	2.53^{h}	21.80^{f}	33.84^{a}
T4-As10ppm + *Glomus mossaea* @0 g/pot	7.86^{fg}	0.45^{a}	9.17^{d}	68.40^{d}	18.69^{f}
T5-As10 ppm+ *Glomus mossaea* @5.0 g/pot	8.13^{de}	0.46^{a}	6.11^{e}	64.10^{de}	28.47^{b}
T6-As10ppm+ *Glomus mossaea* @10.0 g/pot	8.46^{ab}	0.49^{a}	5.93^{e}	59.46^{e}	26.72^{c}
T7-As20ppm + *Glomus mossaea* @0 g/pot	7.76^{g}	0.45^{a}	17.65^{a}	180.00^{a}	16.85^{g}
T8-As20 ppm+ *Glomus mossaea* @5.0g/pot	8.15^{de}	0.48^{a}	13.21^{b}	166.67^{b}	19.22^{e}
T9-As20ppm+ *Glomus mossaea* @10.0 g/pot	8.25^{cd}	0.52^{a}	12.16^{c}	155.33^{c}	24.73^{d}

Values followed by the same small letters do not differ significantly ($p < 0.05$.) between treatments.

Table 3: Pearson correlation between Arsenic uptake in wheat and Glomalin content in soil

Parameters	As in Root	As in leaf	As in Stem	As in Grain	As uptake by Grain	Grain yield	Total Glomalin	AM	Available P in Soil	pH	OC	Available As in Soil	Total As in Soil
As in Root	1												
As in leaf	.917**	1											
As in Stem	.966**	.925**	1										
As in Grain	.954**	.916**	.949**	1									
As uptake by Grain	.926**	.814**	.908**	.949**	1								
Grain yield	-.598**	-.769**	-.635**	-.675**	-.415*	1							
Total Glomalin	-.392*	-.478*	-.422*	-.430*	-.365	.428*	1						
AM	-.215	-.294	-.228	-.261	-.141	.443*	-.133	1					
Available P in Soil	-.849**	-.926**	-.857**	-.864**	-.710**	.886**	.516**	.392*	1				
pH	-.726**	-.878**	-.788**	-.798**	-.602**	.926**	.431*	.375	.900**	1			
OC	-.846**	-.912**	-.886**	-.857**	-.756**	.783**	.495**	.365	.949**	.870**	1		
Available As in Soil	.946**	.841**	.921**	.896**	.883**	-.498**	-.351	-.184	-.744**	-.627**	-.716**	1	
Total As in Soil	.889**	.747**	.851**	.808**	.869**	-.292	-.293	-.098	-.619**	-.453*	-.614**	.964**	1

**Correlation is significant at the 0.01 level and *Correlation is significant at the 0.05 level

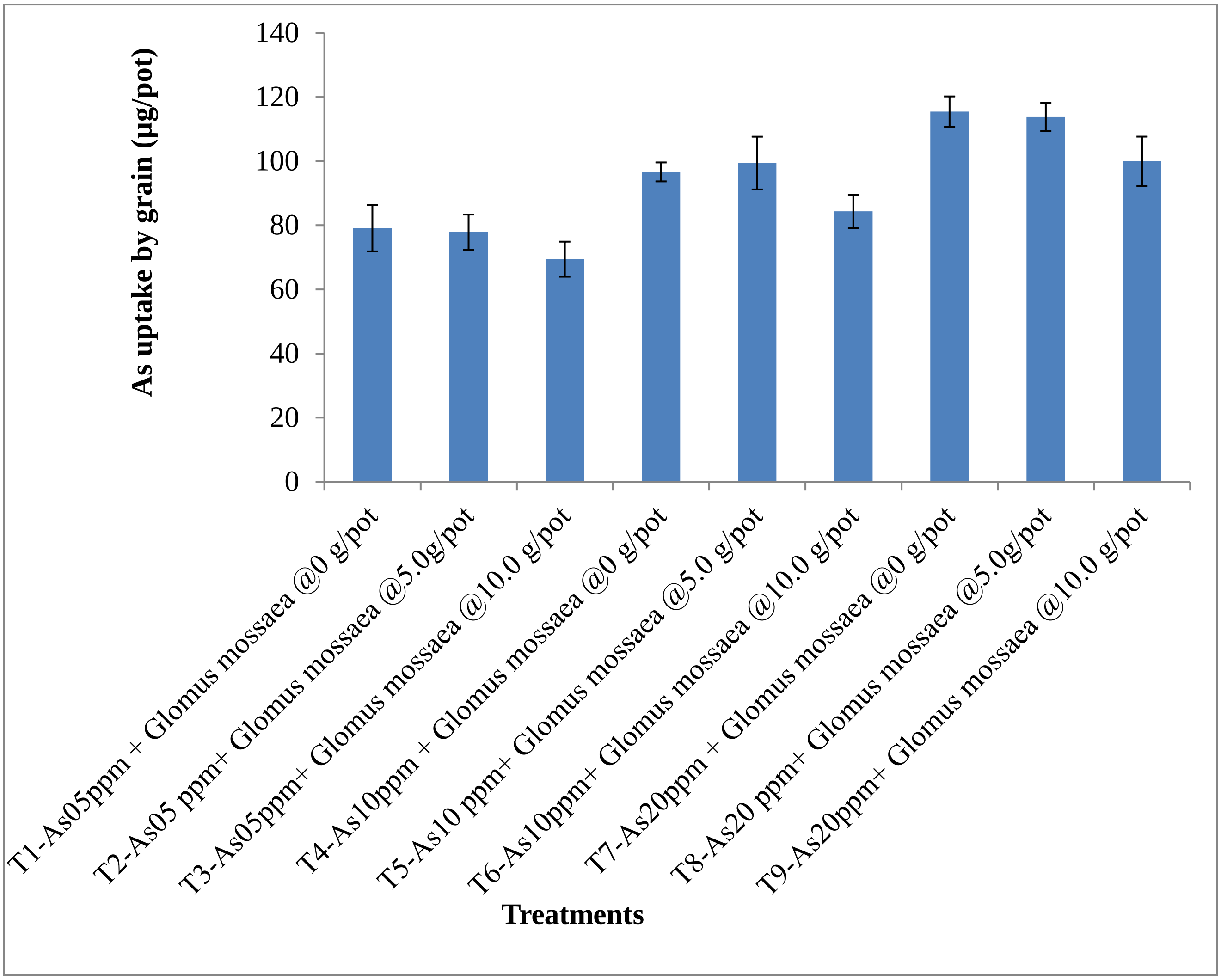

Fig 1: Effect of *Glomus mosseae* on As uptake by wheat grain.