



TRICHODERMA FUNGUS IN MITIGATION OF RHIZOSPHERE ARSENIC: WITH SPECIAL REFERENCE TO BIOCHEMICAL CHANGES

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Abstract

Heavy metal stress is one of the plant stress which is responsible for the reduction in the yield as well as the growth and development of the plants. Arsenic is the fourth most dangerous heavy metal based on the list of heavy metals of the World Health Organisation (WHO). Arsenic stress causes many diseases to human beings when consumed through food, drinking water and by any other sources. Arsenic causes cancer, skin irritations, etc. to the humans. Here *Trichoderma viride* is used to mitigate the heavy metal Arsenic stress. *Trichoderma viride* is a biofertilizer which is used to increase the growth of the plant and helps in the solubilization of the nutrients which are unavailable to the plants. For checking the results of heavy metal stress and the *Trichoderma viride* biochemical are used, biochemical like Membrane stability index, Membrane Injury Index, Total Soluble Proteins, Total Soluble Sugars, Total Phenols, etc. are used. The results of the research work are that *Trichoderma viride* is used well against heavy metal stress.

Keywords: Arsenic, Biochemical, Cancer, Mitigation, Proteins, Stability, Trichoderma.

Introduction

Heavy metals are metals that have a high thickness and are even harmful at low concentrations. Arsenic, Mercury, Chromium, Cadmium, and Lead are some heavy metals (Kumar and Dwivedi, 2018a; Kumar *et al.*, 2018b; Kumar *et al.*, 2018c; Kumar *et al.*, 2018d; Kumar and Purnima, 2018e; Kumar and Pathak, 2019f; Kumar *et al.*, 2019g; Siddique and Kumar, 2018h; Siddique *et al.*, 2018i). These heavy metals are harmful to the human body as well as to plants. Nowadays these heavy metals are increasing day by day in our environment and soil and cause many harmful diseases to humans and pollute the environment as well (Pathak *et al.*, 2017j; Prakash and Kumar, 2017k; Kumar and Mandal, 2014L; Kumar *et al.*, 2014m; Kumar *et al.*, 2014n; Kumar, 2013o; Kumar and Dwivedi, 2015p; Gogia *et al.*, 2014q; Kumar, 2014r; Kumar *et al.*, 2012s). The heavy metals can enter in our environment by agricultural, pharmaceutical, industrialization, human interventions, Excessive or high amount of fertilizers and coal mining also. Heavy metals like Mercury causes Minamata disease in human beings, Lead causes Itai Itai disease, NO₃ causes blue baby syndrome and H₂S causes Akochi disease in rice plant (Mishra *et al.*, 2012t; Kumar *et al.*, 2011u; Kumar *et al.*, 2011v; Kumar and Pathak, 2016w; Pathak *et al.*, 2016x; Kumar *et al.*, 2018y; Kumar *et al.*, 2018z; Kumar *et al.*, 2018aa; Kumar *et al.*, 2018bb; Kumar *et al.*, 2018cc). Arsenic is a semi-metal means it shows both the properties of metals and nonmetals. It belongs to the P block of the periodic table and is a member of the nitro group called Pnictogens and having atomic number 33. As the era of the green revolution started the use of fertilizers and chemicals are also started. These fertilizers provided good yield to the farmers due to which farmers started using an overdose of the chemical fertilizers. This provides good yield but causes a reduction in the fertility of the soil. The overuse or extra dose of fertilizers results in the accumulation of these metals in the soil which

is dangerous for human life. If the limit of arsenic exceeds 70-200mg then it causes Cancer, skin related diseases and diabetes (Kumar, 2018i; Kumar, 2018ii; Kumar, 2018iii; Kumar, 2018iv; Kumar, 2018v; Kumar, 2018vi; Kumar, 2018vii; Kumar, 2018viii; Kumar and Pathak, 2018ix; Kumar and Pathak, 2018x; Kumar and Pathak, 2018xi; Kumar *et al.*, 2018xiii; Kumar and Pathak, 2018xiv; Kumar and Pathak, 2018xv; Kumar and Pathak, 2018xvi; Kumar and Pathak, 2018xvii; Kumar and Pathak, 2018xviii). Arsenic harms soil microorganisms present in the soil. Arsenic is mainly found in two forms that are Arsenic trioxide and Arsenic pentoxide out of which Arsenic Trioxide is more harmful and is mainly available in soils. Bangladesh is the country having the largest amount of arsenic contamination in the soil. The amount of Arsenic that is liable for humans should not exceed from 10ppb. But in the soils of Bangladesh, the amount of arsenic is more than 50ppb which is very harmful to human life and causes Cancer. Here, Trichoderma is used to mitigate arsenic stress. Trichoderma is a biofertilizer and is used to control stresses like drought stress, heavy metal stress and helps in increasing the fertility and productivity of the soil (Kumar and Dwivedi, 2018gg; Kumar *et al.*, 2018ff; Kumar *et al.*, 2018cd; Kumar and Pathak, 2018kk; Kumar and Pathak, 2018pq; Singh *et al.*, 2020a; Singh *et al.*, 2020b; Sood, *et al.*, 2020; Bhadrecha *et al.*, 2020; Singh *et al.*, 2020c; Sharma *et al.*, 2020; Singh *et al.*, 2020d; Bhati *et al.*, 2020; Singh *et al.*, 2019; Sharma *et al.*, 2019).

Methodology

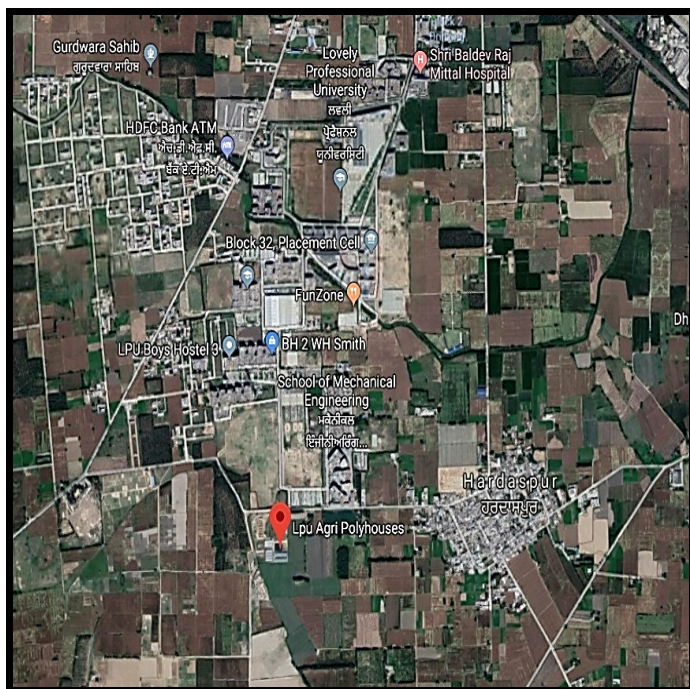
The research experiment was conducted in the open environment of Lovely Professional University, Jalandhar, District-Kapurthala, Punjab. The experiment is a pot experiment and successfully done with the help of the School of Agriculture, LPU. LPU is located about 359.1Km from the capital of India i.e. New Delhi with latitude 31.252232 and longitude 75.703115. Heavy metal stress of Arsenic is

provided through exogenous application of Arsenic Trioxide in the soil and application of biofertilizers, *Trichoderma viride* is also applied through the exogenous application. The pot size is 30x45cm. In each pot, there is 7-10 Kg soil (Kumar P., Pathak S. 2018xv, Kumar P., Pathak S. 2018xvi, Kumar P., Pathak S. 2018xvii, Kumar P., Pathak S. 2018xviii). Arsenic application is applied 2-3 days before sowing. *Trichoderma* application also provides 4-5 days before sowing. The design used is a Completely Randomized Design (CRD) (Figure 1).

Table 1 : Experimental Details

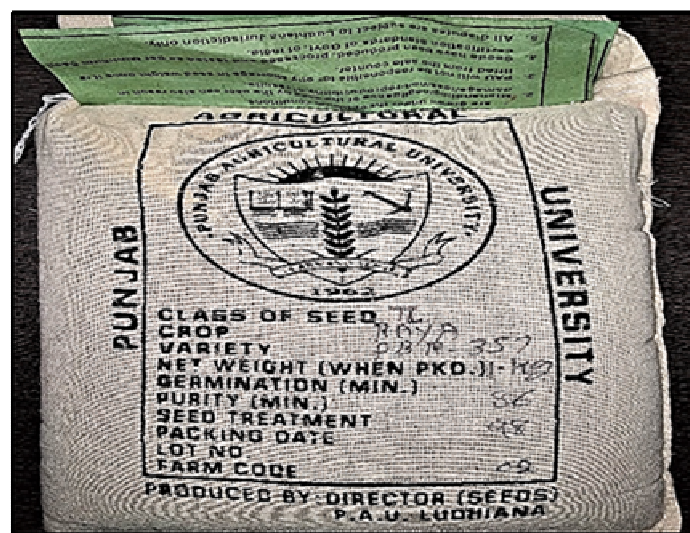
Treatments	Treatment Details
T ₀	Control
T ₁	Arsenic trioxide (50ppm per 10Kg of soil)
T ₂	<i>Trichoderma viride</i> (5 gram per 10Kg of soil)+ Arsenic trioxide (50ppm per 10Kg of soil)
T ₃	<i>Trichoderma viride</i> (5 gram per 10 Kg of soil)

Treatments=4, Replications=3, Design=Completely Randomized Design (CRD)



Source: (Google Map, 2019)

Fig 1. Google photo of LPU (Experiment site)



(Source: Brought by the Authors from PAU Ludhiana for Research work)

Fig 2: S2-PBR 357 Details

Observations to Be Recorded

The observations are recorded from the 15 days plant sample. All standards are used while taking observations. The biochemical is used for taking the observations. The observations are taken based on various protocols of Total Soluble Sugars, Total Soluble Proteins, Total Phenols, Membrane Stability Index and Membrane Injury Index. Various methods used for estimating the biochemical is given in Table 2.

Table 2: Biochemical and Their Methods

Biochemicals	Methods
Total Soluble Sugar	Sadasuvam and Manickam,1992
Total Soluble Protein	Bradford Method,1976
Total Phenols	Mahadevan and Sridhar,1982
Membrane Stability Index	Sullivan Method,1972
Membrane Injury Index	Sullivan Method,1972

Results and Discussion

1. Total Soluble Sugars (TSS): In 1992, Sadasuvam and Manickam suggested a protocol for the estimation of Total Soluble Sugars (TSS), their protocol is used in the research. In the experiment, a high amount of sugars are present in T₃ treatment that is of *Trichoderma viride* and the least amount of sugars are present in T₁ treatment that is of Arsenic trioxide (Figure 1).

Climatic Conditions

In Phagwara, the temperature of summers ranges between 35^oC to 45^oC and in winters the temperature ranges between 12^oC to 15^oC. The area of Lovely Professional University lies between central plains zones of Northeast regions of India. The annual rainfall of Phagwara is 200-1000mm/year. The temperature of LPU mainly as of Jalandhar city and Kapurthala district but sometimes it varies. In the Punjab region, the highest temperature recorded is 48^oC. The temperature needed for the growth of the mustard crop is 10^oC to 25^oC. This temperature is available in November in Punjab area which is sufficient for the growth of the Mustard crop (Fig 2).

Treatments Details

The treatments for this research are four in number. The genotype for the research was taken from the Seed store of Gate no.1 having farm code 02, Punjab Agricultural University, Ludhiana. The genotype of Mustard (Raya) is PBR-357 having germination percentage 86% and purity percentage 98%. The four treatment control, Arsenic, *Trichoderma viride* + Arsenic and *Trichoderma viride*. Four treatments (T₁, T₂, T₃, and T₄) are there with three replications R₁, R₂, and R₃. *Trichoderma viride* treatment is taken at 150 spores colonies per count per Kg of soil taken with the help of Haemocytometer that is 5gram per 10 Kg of soil. Arsenic trioxide is taken at 0.5gram per 1litre of water in 10 Kg of soil that is 50ppm for one-pot (Table 1).

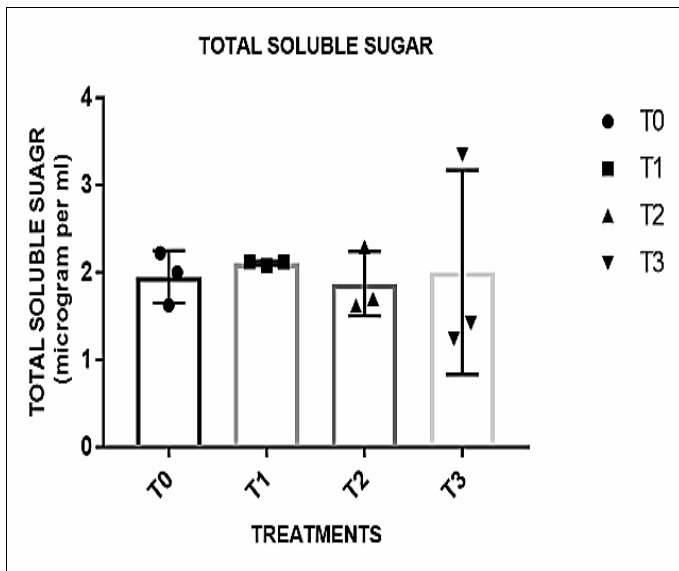


Fig. 1: Total Soluble Sugar

where, values are mean +SD, T₀: Control, T₁: Arsenic trioxide (50ppm per 10kg of soil), T₂: *Trichoderma viride* (5 gram per 10kg of soil)+ Arsenic trioxide (50ppm per 10kg of soil), T₃: *Trichoderma viride* (5 gram per 10 kg of soil)

2. Total Soluble Protein (TSP): For the estimation of Total Soluble Proteins (TSP) Bradford Method is used which is given in 1976. In the experiment, the high amount of Total Soluble Proteins are present in the T₂ treatment that is of *Trichoderma viride* + Arsenic trioxide and the least amount of Protein is present in T₁ treatment that is of Arsenic trioxide (Figure 2).

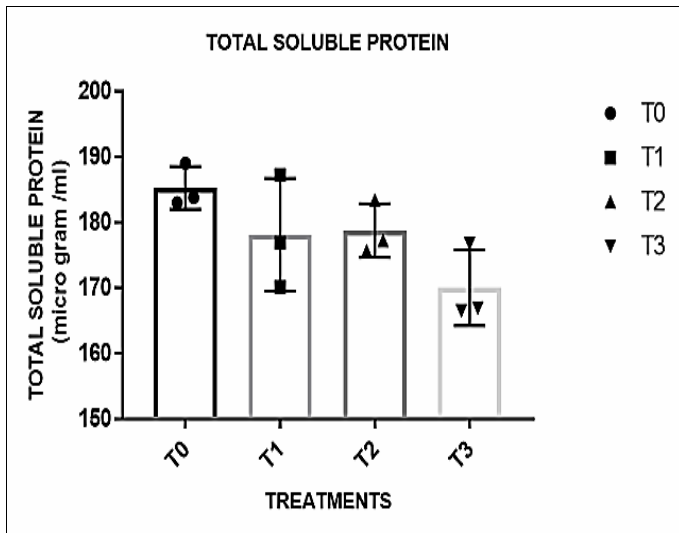


Fig. 2: Total Soluble Protein

where values are mean +SD, T₀: Control, T₁: Arsenic trioxide (50ppm per 10kg of soil), T₂: *Trichoderma viride* (5 gram per 10kg of soil)+ Arsenic trioxide (50ppm per 10kg of soil), T₃: *Trichoderma viride* (5 gram per 10 kg of soil)

3. Total Phenols: For Phenols estimation the protocol of Mahadevan and Sridhar, 1982 is used. If there is more stress the production of Phenols is also high. As per the research experiment, the Total Phenols are present in T₂ treatment that is of *Trichoderma viride* + Arsenic Trioxide in a high amount as compared to others and the least number of Phenols are present in T₁ treatment that is of Arsenic Trioxide (Figure 3).

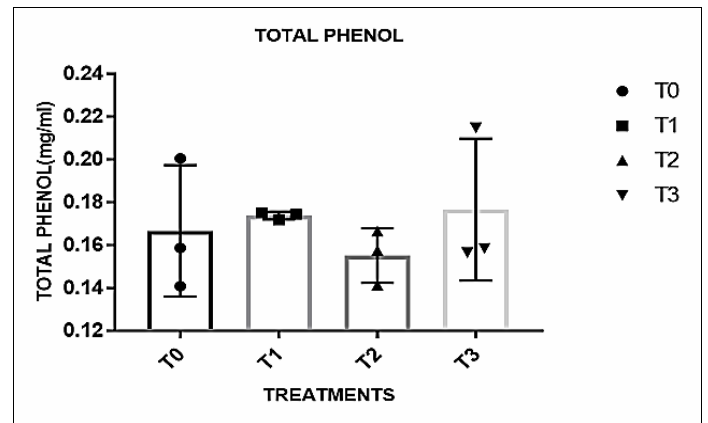


Fig. 3 : Total Phenol

where values are mean +SD, T₀: Control, T₁: Arsenic trioxide (50ppm per 10kg of soil), T₂: *Trichoderma viride* (5 gram per 10kg of soil)+ Arsenic trioxide (50ppm per 10kg of soil), T₃: *Trichoderma viride* (5 gram per 10 kg of soil)

4. Membrane Stability Index (MSI): For the estimation of the Membrane Stability Index (MSI) Sullivan Method (1972) is used. As the amount of metals increases or in high-stress conditions, the stability of the membrane is less. The membrane stability index of treatment T₃ is more that is the treatment of *Trichoderma viride* has more stability than others. And as well the treatment which shows less stability in treatment T₁ that is of Arsenic Trioxide, means Arsenic causes much damage to the plants (Figure 4).

5. Membrane Injury Index (MII): Sullivan Method (1972) is used for membrane injury level estimation. As the level of stress increases then the injury level of the membrane also increases. The treatment T₁ shows more injury than others that means Arsenic Trioxide causes more injury to the plants and causes stress conditions. And the least injury to the plants is caused by the treatment *Trichoderma viride* that is treatment T₃. This shows that the *Trichoderma viride* can be used to mitigate arsenic stress because it causes less injury to the mustard plants (Figure 4).

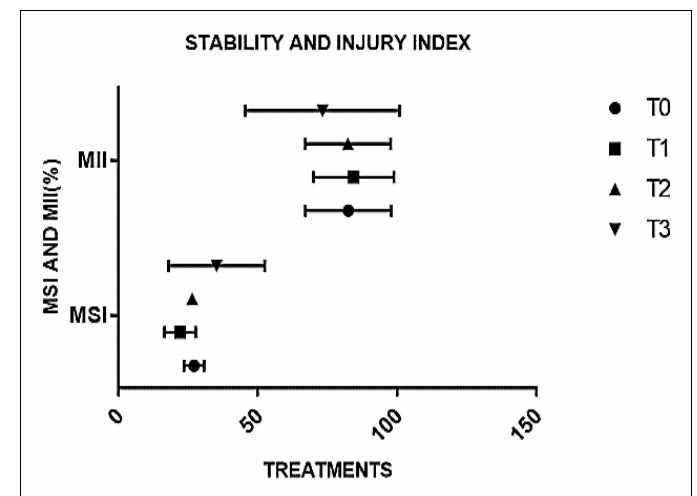


Fig. 4 : Membrane Stability and Injury Index

where, values are mean +SD, T₀: Control, T₁: Arsenic trioxide (50ppm per 10kg of soil), T₂: *Trichoderma viride* (5 gram per 10kg of soil)+ Arsenic trioxide (50ppm per 10kg of soil), T₃: *Trichoderma viride* (5 gram per 10 kg of soil)

Conclusion

Arsenic is harmful to the plants as it shows negative effects on the experiment. As per the whole research, the results of the experiment show that *Trichoderma viride* performs well in all aspects as compared to others. The heavy metal stress caused by the Arsenic trioxide can be mitigated by *Trichoderma viride* because it causes no harm to the plant or causes no stress in plants whereas used to overcome the stress conditions. So, as per the experiment, we recommend that in Arsenic stress conditions *Trichoderma viride* is applied in the soil to overcome that problem and to improve plant growth and yield as well.

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Author Contributions

The study was designed by P.K. and P.K. the biochemical protocolizations were established, the experiment was carried out and the data analyzed and interpreted were collected. The paper has been written by P.K. and P.K.

Conflict of Interest Statement

The authors state that they have no interest in conflicts.

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