



EVALUATION OF ANTIMONY INDUCED BIOCHEMICAL SHIFT IN MUSTARD

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Abstract

Due to various anthropogenic emission activities, antimony (Sb) concentration is gradually rising day by day in the environment. The intake of Sb is more on those soils which are having high contamination in the form of soil soluble Sb. Antimony is a lustrous gray metalloid that is to be present in nature in the form of Sulfide mineral stibnite (Sb₂S₃). Sb is a toxic trace element and it has wider scopes in terms of research as there is very less work is done on the soil contaminated with metalloids like antimony, arsenic, etc. Antimony is found to be less toxic when compared to other elements like arsenic. During our research crop trial, we apply the salt of Antimony potassium tartrate hemihydrate (100 ppm) in solution form individually and also in combination with *Trichoderma hamatum*. 6 gram of *Trichoderma hamatum* is applied in the pot. Various parameters viz; Total soluble sugar, Total soluble protein, Chlorophyll index, Membrane injury index and Membrane stability index are taken into account to check the effect of Sb induced pot culture on Indian mustard (*Brassica juncea*).

Keywords: Anthropogenic, Contamination, Metalloid, Index, Sugar, Injury, Stability.

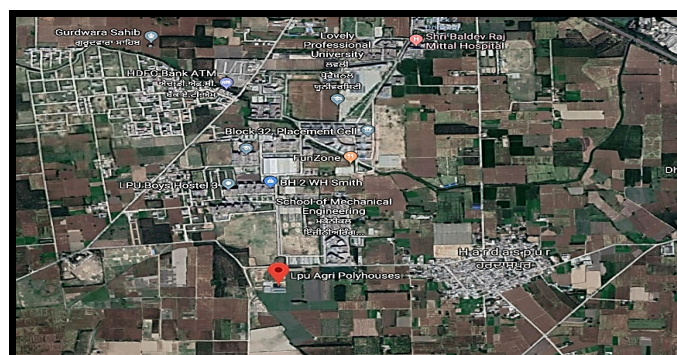
Introduction

Antimony is one of the prevalent toxic heavy metals present in an inorganic form in the different soil flora and can induce toxicity either directly or indirectly to both the plant and animal species (Kumar and Dwivedi, 2018a; Kumar *et al.*, 2018b; Kumar *et al.*, 2018c; Kumar and Dwivedi, 2018d; Kumar *et al.*, 2018e; Kumar and Pathak, 2019f; Kumar *et al.*, 2019g; Siddique and Kumar, 2018h; Siddique *et al.*, 2018i). Though even it is not a requisite provider of nutrients for plant species, yet its amalgam may have toxic effects on plants. Antimony, having various toxicity levels are dependent upon their valence state and the trivalent antimony tends to be more toxic as compared to the pentavalent antimony (Pathak *et al.*, 2017j; Prakash and Kumar, 2017k; Kumar and Mandal, 2014L; Kumar *et al.*, 2014m; Kumar *et al.*, 2014n; Kumar, P. 2013o; Kumar and Dwivedi, 2015p; Gogia *et al.*, 2014q; Kumar, 2014r; Kumar *et al.*, 2012s). Antimony and its amalgam posses cancer-causing hazards and threshold toxicity. Because of the extreme toxicity, antimony was considered as a major pollutant in the environment. During the current century, due to the increased mine digging practices, more industrialization and the advent of a more technological approach in the agricultural systems, there is an increase in environmental pollution worldwide. Metalloids like antimony are one of the biggest sources of environmental pollution (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). To check the effect of Sb toxicity (100ppm) we select the mustard (Genotype PBR-357) and also the application of the *Trichoderma hamatum* (6 g per 10 kg of soil). Different combinations of treatments are applied in a pot having a size (30cm in diameter and 25 cm in height) at a lovely professional (Kumar and Dwivedi, 2018gg; Kumar *et al.*, 2018ff; Kumar 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). The main aim of our experimental trial is to study is the effect of Sb stress on the biochemical behavior of mustard. In this pot trial, Indian mustard grown in soil treated with either a mixture of Sb with *Trichoderma hamatum* or the treatments of Sb and *Trichoderma hamatum* is applied alone as a single treatment. The application of *Trichoderma hamatum* in combination helps to counteract the effect of Sb stress on mustard crop (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).

Materials and Methods

The crop research trial was performed in pots having natural conditions, at the School of Agriculture, Lovely Professional University (LPU), Phagwara, Punjab. The experimental area is situated at an altitude of 232 meters above mean sea level, having latitude and longitude 31.244604 N and 75.701022 respectively (Figure 1).



(Source:- Google Earth, 2020)

Fig.1: Google photo of the experimental site

Climatic Conditions

Punjab (Phagwara region) falls in central plain zones and situated in the Northeastern part of India. The annual rainfall ranges in Punjab in between (250-1000 mm/years). In winter temperature at night time falls to 5 degrees and in the morning the temperature is around 12-15 degrees. In summer the maximum temperature is above 40 degrees Celsius. Ludhiana district of Punjab recorded the highest temperature of 46.1 degrees Celsius with Amritsar and Patiala district recorded 45-50 degrees Celsius temperature in summers. The average annual temperature is 24.1 degrees Celsius with an average annual rainfall of 686 mm. (Source:- en.climate-data.org)

Treatments Details

The pot experimental research trial was performed on the genotype of Mustard PBR-357 which was brought from the Punjab Agriculture University, Ludhiana. The seeds and their details are given in figure 1. Four treatments were taken i.e. (T0, T1, T2, and T3) with three replications (R1, R2, and R3). The total number of pots was 12 having dimensions like diameter and height is 30cm and 25cm respectively. The experiment was conducted on agriculture farm, School of agriculture, Lovely Professional University. Heavy metal toxicity is created by the application of Antimony potassium tartrate hemihydrate 100 ppm per 10 kg of soil and the *Trichoderma hamatum* 6 gram per pot was taken as a treatment. All the treatments are provided to the soil not directly to plants before 2 days after sowing. For estimation of different biochemical samples was taken 15 days after sowing (Table 1 and 2).

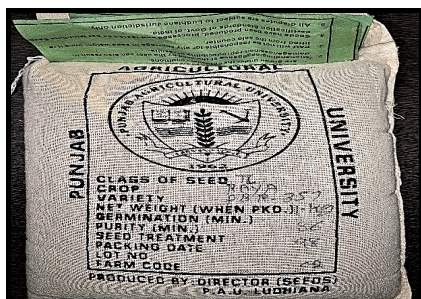


Fig. 1: PBR-357 taken for research

Table 1: Treatments Details

Treatments	Details of the treatments	Time of application
T-0	Control	Before sowing
T-1	Sb + <i>Trichoderma hamatum</i> (100 ppm + 6 g per 10 kg of soil)	Before sowing
T-2	Sb (100 ppm per 10 kg of soil)	Before sowing
T-3	<i>Trichoderma hamatum</i> (6 g per 10 kg of soil)	Before sowing

Table 2: Layout Details

S. No.	Particulars	Details
1.	Layout	CRD
2.	Treatment	4
3.	Replication	3
4.	Total number of pots	4*3= 12
5.	Soil per pot	10 Kg
6.	Genotype	PBR-357

Observation to be recorded

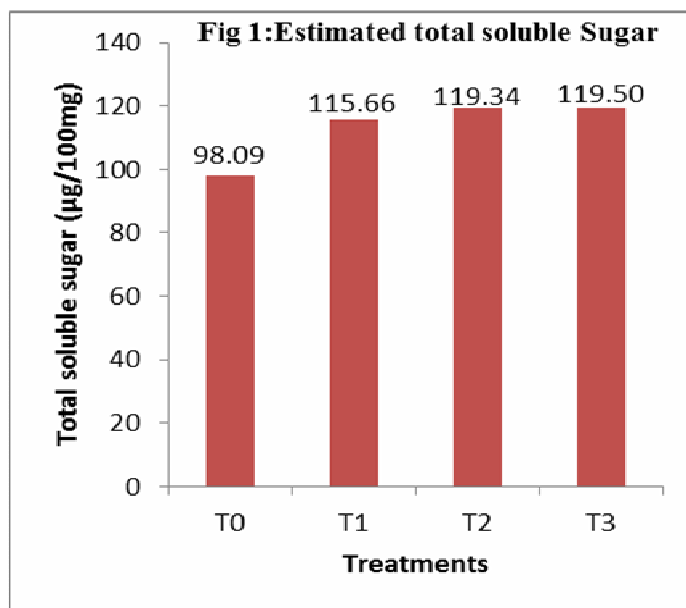
The observation was recorded at 15 days after sowing (DAS). To estimate the biochemical's of different recorded observations, the Standard procedure adopted are given below:

1. **Total Soluble Sugar:** It is estimated by the method proposed by Sadasuvam and Manickam (1992).
2. **Total Soluble Protein:** It is estimated by the method proposed by Bradford, (1976).
3. **Total Phenols:** For the estimation of total phenol the protocol of Mahadevan and Sridhar (1982) is followed.
4. **Chlorophyll Index:** SPAD meter is used for the measurement of the Chlorophyll index.
5. **Membrane injury index(MII) and Membrane stability index (MSI):** Sullivan, C. Y., 1971

Results and Discussion

1. Total Soluble Sugar (TSS)

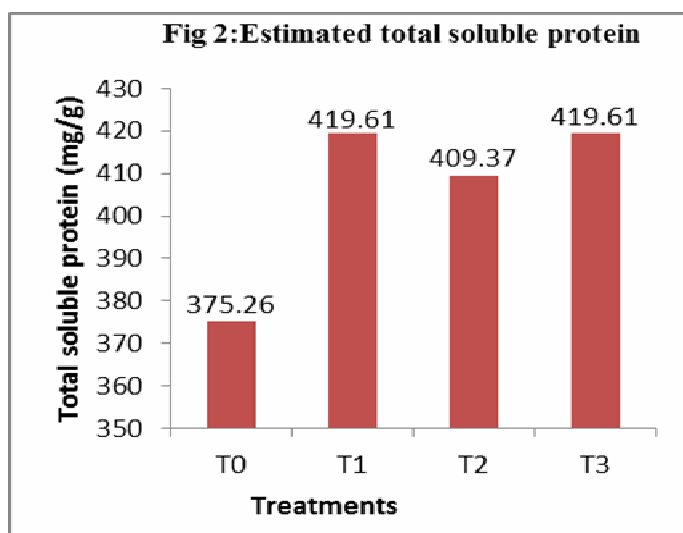
For the estimation of TSS, Sadasuvam and Manickam method are used which was given in the year 1992. When T2 is compared with other treatments then there is a 0.14 % increase in T3 whereas there is 21.66 % and 3. 18 % decrease in T0 and T1. From these results, we conclude that in the case of T3 there is more percentage of production of total soluble proteins as compared to other treatments.



where, T0: Control, T1: Sb + *Trichoderma hamatum* (100 ppm + 6 g per 10 kg of soil), T2: Sb (100 ppm per 10 kg of soil), T3: *Trichoderma hamatum* (6 g per 10 kg of soil)

2. Total Soluble Protein (TSP)

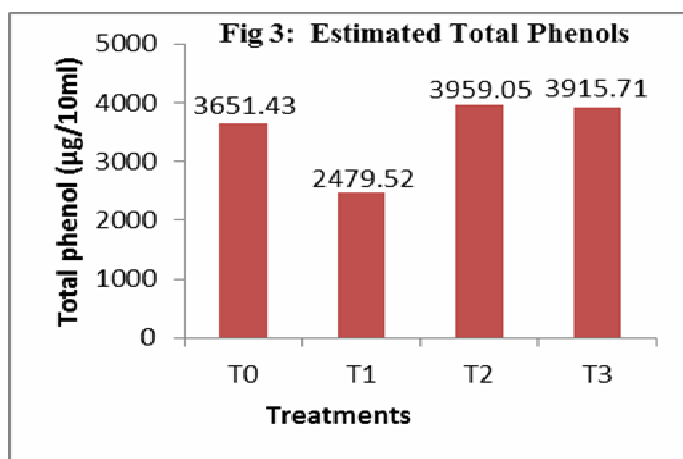
For the estimation of TSP, the Bradford method is used which was given in the year 1976. When we compare T2 with other treatments, there is a 2.44% more increase in both T1 and T3 and a 9.09% decrease in the amount of TSP. Thus both T1 and T3 have more amount of total soluble protein as compared to other treatments.



where, T0: Control, T1: Sb + *Trichoderma hamatum* (100 ppm + 6 g per 10 kg of soil), T2: Sb (100 ppm per 10 kg of soil), T3: *Trichoderma hamatum* (6 g per 10 kg of soil)

3. Total Phenols

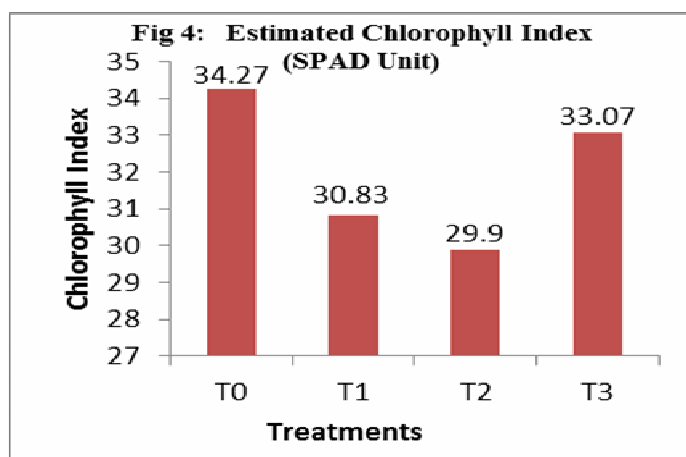
Mahadevan and Sridhar's (1982) protocol was followed for phenol production. As we know that in stress conditions plants produce more amount of phenols. Here also there is maximum production in T2 and there is 8.42%, 59.67% and 1.11% decrease in T0, T1, and T3 respectively. Hence, we can conclude that in the presence of *Trichoderma hamatum* along with the antimony, there is less amount of phenol production means it can mitigate the stress of Sb for a certain level.



where, T0: Control, T1: Sb + *Trichoderma hamatum* (100 ppm + 6 g per 10 kg of soil), T2: Sb (100 ppm per 10 kg of soil), T3: *Trichoderma hamatum* (6 g per 10 kg of soil)

4. Chlorophyll Index

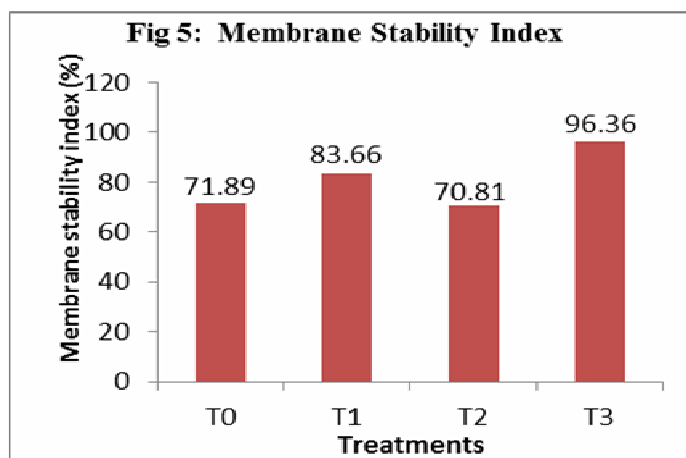
It is measured with the help of SPAD (Soil Plant Analysis Development) chlorophyll meter. For this, we compare T2 with other treatments. During the comparison it is found that T2 has less chlorophyll index whereas T0, T1, T3 has 12.75%, 3.02% and 9.58% more chlorophyll index than T2. The better result is obtained from T0 followed by the T3 (Figure 4).



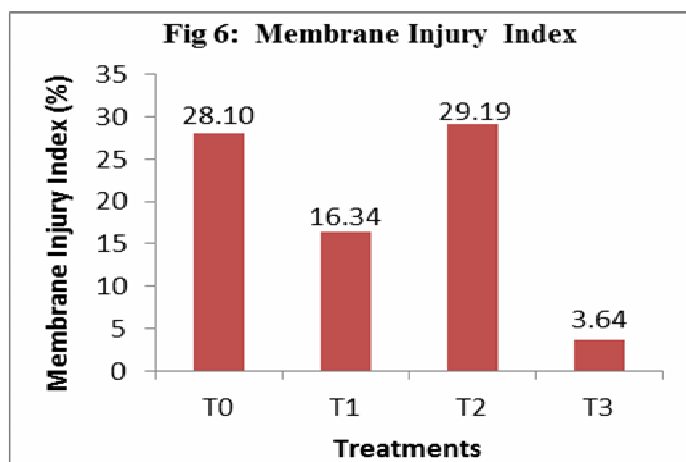
where, T0: Control, T1: Sb + *Trichoderma hamatum* (100 ppm + 6 g per 10 kg of soil), T2: Sb (100 ppm per 10 kg of soil), T3: *Trichoderma hamatum* (6 g per 10 kg of soil)

5 Membrane stability index (MSI) and Membrane injury index (MSI):

For membrane stability, T3 shows better results followed by the T1 i.e. 36.08% and 18.15% respectively as compared with T2. Therefore, we can say that in the presence of *Trichoderma hamatum* membrane stability shows better results. On the other hand, the Membrane injury index T2 compared with other treatments then there is 3.73%, 44.02%, and 87.53 % less in MII compared to T2. Hence we can say the there is a significant amount of decrease in MII in the presence of VAM (Figures 5 and 6).



where, T0: Control, T1: Sb + *Trichoderma hamatum* (100 ppm + 6 g per 10 kg of soil), T2: Sb (100 ppm per 10 kg of soil), T3: *Trichoderma hamatum* (6 g per 10 kg of soil)



Conclusion

Antimony is one of the prevalent toxic heavy which does not have any significant role in plant physiology. The presence of Sb either in the plant as well as in soil affects plant growth and development. In plants, it affects plant metabolism and reduces the plant cell homeostasis and creates stressful conditions for plant growth. These stressful conditions disturb the plant's biochemical reactions going inside their body. Therefore, to counteract the effect of Sb stress on the plant a small pot study concludes that the general application of *Trichoderma hamatum* to soils is proved beneficial. Thus we can apply *Trichoderma hamatum* in those soils that affect heavy metal toxicity like Sb to improve the plant growth and their development.

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Author Contributions

The study was designed by C. M. 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 J.C.

Conflict of Interest Statement

The authors state that they have no interest in conflicts.

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