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## Biochemical shift of mustard grown under cadmium contaminated soil

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**Abstract**

Cadmium is silvered colored poisonous transitional metal having atomic number 48. In-plant, Cd has no role in biological as well as physical functions but its entry is can't be stopped by the plant roots. The plant has its defense system to control the entry hazardous elements but somehow Cd makes its path to enter into the plant. Cadmium was discovered in 1817 by Friedrich. Stromeyer and Karl Samuel Leberecht Hermann in Germany. Cadmium lies in the category of non- threshold elements which means that only the presence of cadmium will affect the plant growth. Therefore, to check the effects of cadmium on the plant growth we target the different biochemicals of plants viz; Total soluble sugar, Total soluble protein, Chlorophyll index, Membrane injury index and Membrane stability index. After the estimation of these biochemicals, we found that there is a significant when compared with control and other treatments. Cadmium toxicity is created in soil by the application of Cadmium sulfate (70ppm). To mitigate the effect of cadmium the application 150 spores of VAM per kg of soil in the pot. Hence the presence of VAM proved good for the plant because it helps in mitigating the effect of cadmium at a certain level.

**Keywords:** Atomic, biological, cadmium, chlorophyll, element, growth, hazardous, injury membrane, mitigating, stability

**Introduction**

Before the 1960's Indian agriculture depends upon the organic source of fertilizers like FYM, Goat and sheep dropping, Crop residues, Green leaf manure, and ash is also used as a source of the nutrient (Kumar P. 2018i., Kumar P. 2018ii., Kumar P. 2018iii, Kumar P.2018iv, Kumar P. 2018v., Kumar P. 2018vi) <sup>[5, 36, 37, 38, 39, 44]</sup> When the green revolution takes in the 1960s to feed the day by day increasing population there was used of fertilizers take place mainly nitrogenous fertilizers and high yielding dwarf varieties, hybrids take place (Kumar, P., Dwivedi, P. (2018a) <sup>[23]</sup>, Kumar, P., Kumar S. *et al.* (2018b) <sup>[24]</sup>, Kumar, P., Misao, L., *et al.*, 2018c <sup>[25]</sup>, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al.*, 2018e <sup>[1]</sup>, Kumar, P. Pathak, S. 2019f <sup>[2]</sup>, Kumar, P. Siddique, A. *et al.*, 2019g) <sup>[3]</sup>. Hybrids are very good at the response to the application of synthetic fertilizers as well as other agronomic practices also. Initially, farmers as forced to use the chemical fertilizers but when the application of synthetic fertilizers shows much better results as compared to without fertilizers. As the application of fertilizer shoes, better results than farmers willing to used chemical fertilizers without knowing that these chemical fertilizers also act as a source of other pollutants like Cadmium. In-between (1960-2000) there is a massive increase in the production but afterward yield id declining years after years because of a decrease in the organic matter in the soils and increase in the other contaminants. Cadmium was the first time used as a substitute for tin and as a pigment by the paint industries at the time of world war first. The main source of cadmium is excessive use of fertilizers, mining and smelting industries, presence sewage and sludge, etc. At present cadmium is used in rechargeable batteries and also present in the tobacco smoke which pollutes the environment (Kumar P. 2018vii, Kumar P. 2018viii, Kumar P., Pathak S. 2018ix, Kumar P., Pathak S. 2018x, Kumar P., Pathak S. 2018xi, Kumar P., Pathak S, Kumar P., Pathak S. 2018xiii, Kumar P., and Pathak S. 2018xiv) <sup>[42, 43, 44, 45, 46, 47, 48]</sup>. Cd also affects the humans by causing the Itai- Itai disease (i.e. loosening in the strength of bones). To check the effect of toxicity of cadmium (70ppm) we select the mustard (Genotype PBR-357) and also the application of the VAM 150 spores per kg of soil. Different combinations as treatments are grown in a pot having a size (30cm in diameter and 25 cm in height) at a lovely professional university (Kumar P., Pathak S. 2018xv, Kumar P., Pathak S. 2018xvi, Kumar P., Pathak S. 2018xvii, Kumar P., Pathak S. 2018xviii) <sup>[50-53]</sup>. The main objective of my study is look what is the effect of Cd stress on the biochemical of mustard though mustard also possess the capacity

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to grow in toxic soils and the application of VAM in combination also helps in mitigating the effect of Cd stress on plant reason behind that VAM posses the potential to trap the heavy metals like Cd in soils and don't allow to go within the plant (Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i, Pathak, S., Kumar, P., P.K Mishra, M. Kumar, M. 2017j, Prakash, A., P. Kumar, 2017k., Kumar, P., Mandal, B., 2014L, Kumar, P., Mandal, B., Dwivedi P., 2014m., Kumar, P., Kumar, P.K., Singh, S. 2014n, Kumar, P. 2013o, Kumar, P., Dwivedi, P. 2015p, Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q) [4, 36, 6-13].

### Methodology

The experiment was conducted 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).

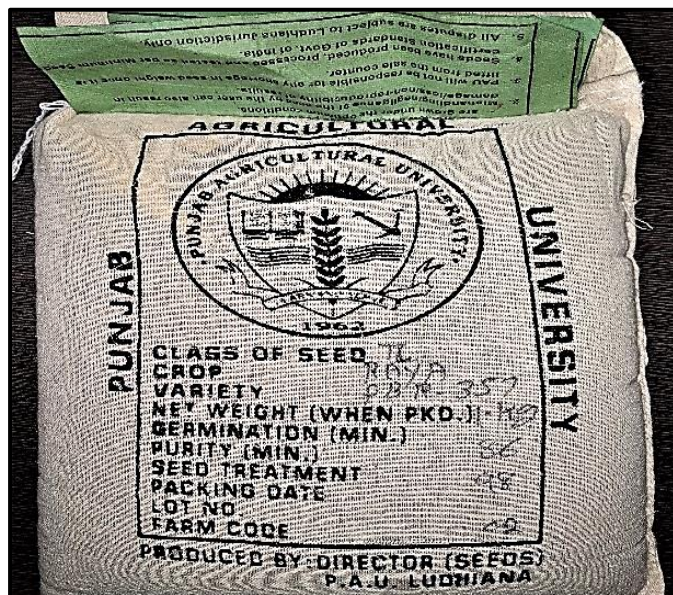


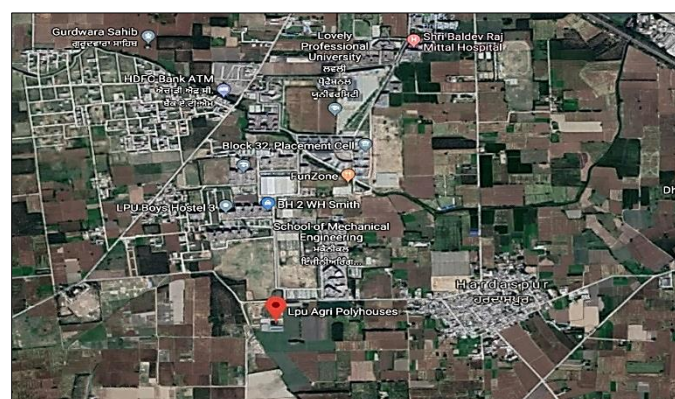
Fig 2: PBR-357 taken for research

Table 1: Treatments Details

Treatments	Details of the treatments	Time of application
T-0	Control	Before sowing
T-1	VAM (150 spores per kg of soil)	Before sowing
T-2	Cd (70ppm per 10 kg of soil)	Before sowing
T-3	Cd + VAM (70 ppm + 150 spores)	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



Source: Google Earth, 2020

Fig 1: Google photo of the experiment 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.

### Treatments Details

The pot experiment was conducted on one genotype of Mustard PBR-357 took from the Punjab Agriculture University Ludhiana. The seeds and their details are given in figure 1. Three replication and four treatments were taken i.e. (T0, T1, T2, and T3) with replication (R1, R2, and R3). The total number of pots was 12 having dimensions like diameter and height is 30cm and 25cm respectively. Experimented was conducted on agriculture farm, School of agriculture, Lovely Professional University. Heavy metal toxicity is created by the application of Cadmium sulfate 70 ppm per 10 kg of soil and the VAM (Vesicular Arbuscular Mycorrhiza) 150 spores per kg of soil 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).

### Observation to be recorded

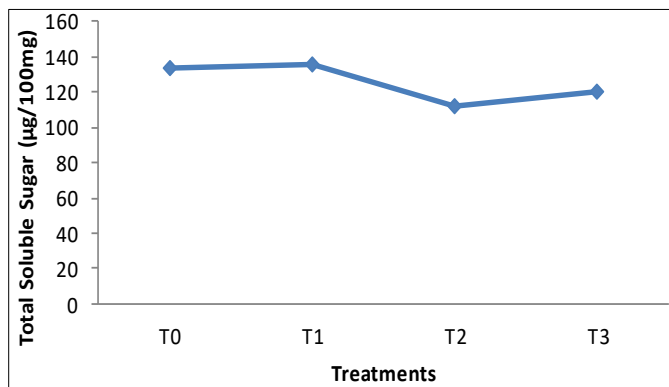
The observation was recorded at 15 days after sowing (DAS). To estimate the biochemical's of different recorded observations, the Standard procedure adopted are given below:

- Total Soluble Sugar:** It is estimated by the method proposed by Sadasuvam and Manickam (1992).
- Total Soluble Protein:** It is estimated by the method proposed by Bradford, (1976).
- Total Phenols:** For the estimation of total phenol the protocol of Mahadevan and Sridhar (1982) is followed.
- Chlorophyll Index:** SPAD meter is used for the measurement of the Chlorophyll index.
- 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 is used given in 1992. The results are if T2 is compared with other treatments then there is 16.7033% more total soluble protein in the case of T0, 17.55085% in the case of T1 and 7.445% in T3. From this, we conclude that in the case of T1 there more percentage of production of total soluble proteins as compared to other treatments.

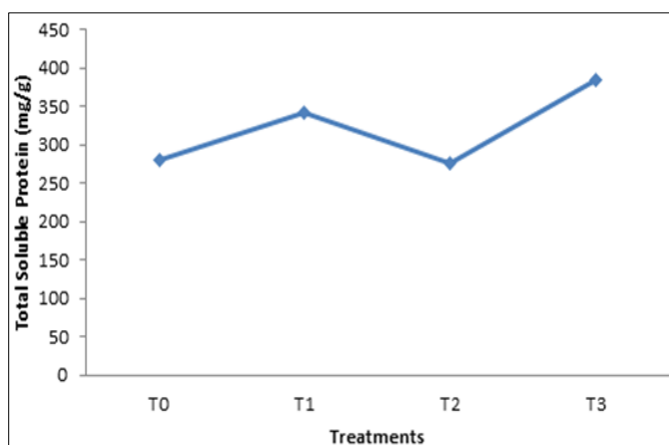


**Fig 1:** Estimated total soluble Sugar

where, T0: Control, T1: VAM (150 spores per kg of soil), T2: Cd (70 ppm per 10 kg of soil), T3: Cd + VAM (70 ppm + 150 spores)

## 2. Total Soluble Protein (TSP)

In the case of the TSP, the Bradford method was used for the estimation purpose. If we compared T2 with other treatments there is 1.21%, 19.40%, 28.31% more amount of TSP in T0, T2, and T3 respectively. Thus T3 has more amount of total soluble protein as compared to other treatments.

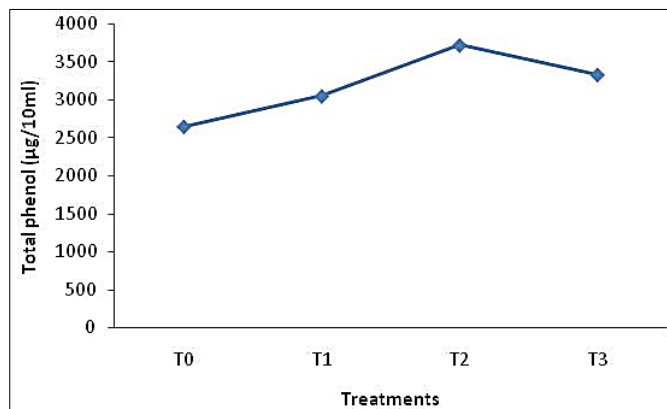


**Fig 2:** Estimated total soluble protein

Where, T0: Control, T1: VAM (150 spores per kg of soil), T2: Cd (70 ppm per 10 kg of soil), T3: Cd + VAM (70 ppm + 150 spores)

## 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. In this experiment plant of treatment, T2 produces 40.55%, 21.97%, and 11.68% more amount phenol compared with T0, T1, and T3 respectively. Hence, we can say that in the presence of VAM there is less amount of phenol production means it can mitigate the stress of Cd for a certain level.

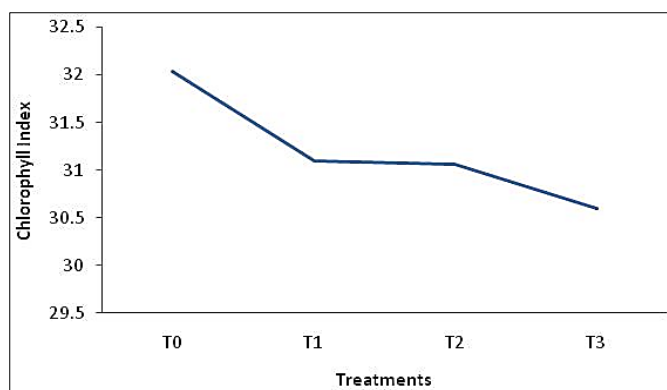


**Fig 3:** Estimated Total Phenols

Where, T0: Control, T1: VAM (150 spores per kg of soil), T2: Cd (70 ppm per 10 kg of soil), T3: Cd + VAM (70 ppm + 150 spores)

## 4. Chlorophyll Index

It is measured with the help of SPAD (Soil Plant Analysis Development) chlorophyll meter. For this if we compare T2 with other treatments than T0 is 2.91%, T1 is 0.107% and T3 is about 1.525% more chlorophyll index. The better result is obtained from T0 followed by the T3 (Figure 4).



**Fig 4:** Estimated Chlorophyll Index (SPAD Unit)

where, T0: Control, T1: VAM (150 spores per kg of soil), T2: Cd (70 ppm per 10 kg of soil), T3: Cd + VAM (70 ppm + 150 spores)

**5 Membrane injury index (MII) and Membrane stability index (MSI):** For membrane stability, T1 shows better results followed by the T3 i.e. 96.742% and 96.428% respectively as compared with T2. Therefore, we can say that in the presence of VAM membrane stability shows better results. On the other hand, the Membrane injury index T2 compared with other treatments then there is 4.249% increased in MII compared to T0, 12.105% more in case of T1 and 10.888% more in T3. Hence we can say the there is a significant amount of decrease in MII in the presence of VAM (Figures 5 and 6).



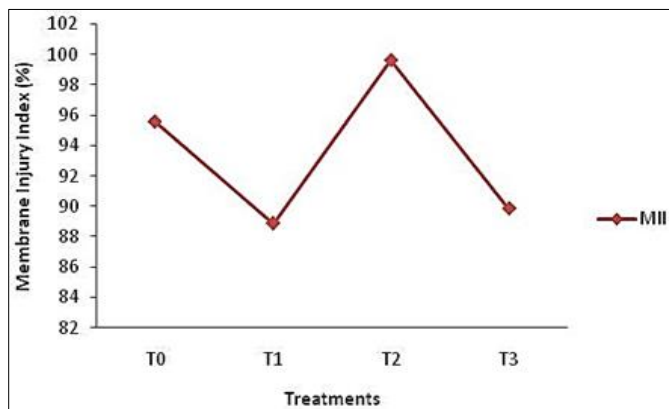


Fig 5: Membrane Injury Index

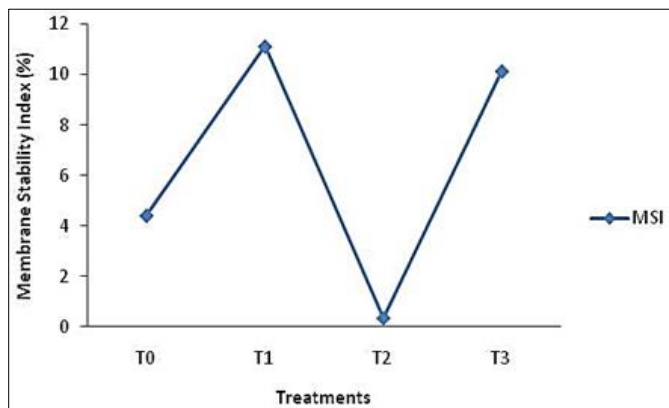


Fig 6: Membrane Stability Index

Where, T0: Control, T1: VAM (150 spores per kg of soil), T2: Cd (70 ppm per 10 kg of soil), T3: Cd + VAM (70 ppm + 150 spores)

### Conclusion

Cadmium is a known threshold element for the plant has no significant role in plant metabolism. The presence of Cd either in the plant as well as in soil affects the plant growth in both the ways. In plants, it affects plant metabolism and reduces the plant cell homeostasis and result in the production of ROS species. The production of various ROS species disturbs the plant biochemical reaction going inside the plant. Therefore, to mitigate the effect of Cd stress on the plant a small pot study concludes that the general application of VAM to soils is proved beneficial. Thus we can apply VAM in those soils affect with heavy metal toxicity like Cd to improve the plant growth.

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### Author Contributions

The study was designed by P. K. and J.C. 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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