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Enhancement effect of biofertilizers on germination percentage and plant height in maize grown under chromium toxic soil

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Abstract
Due to different activities by the human being like ore extraction and application of different processes causing the heavy metal mobility which leads to the addition of these elements in the environment. As we all know that nature of heavy metal is non- biodegradable hence accumulating in the environment and entering the food chain causing the contamination. These type of contamination having environmental risk as well as affecting the health of human. Heavy metal is mutagenic, endocrine, carcinogenic and teratogenic in nature which causes a neurological problem, especially in children. By considering all these points in mind remediation of these heavy metal is important to have a safe environment for survival. There are different methods for the remediation of heavy metal which are having the many limitations that are alteration of soil properties, high cost, disturbance in soil microflora and high demand labour. The present research work was carried out during the Zaid season in the Department of Agronomy as a pot culture experiment in the farm, School of Agriculture, Lovely Professional University, Phagwara. A pot experiment was set up with *Zea mays*, as test plant to evaluate the role of trichoderma, rhizobium and mycorrhiza mediated mitigation of chromium toxicity on morphological parameters. The result indicates that exposed plants to chromium toxicity (T1) showed the reduction in plant growth which includes a decrease in germination percentage by 33.32% as compared to control treatment (T0). The result indicates that average plant height was significantly decreased by 23.29%, 14.28% and 18.65% when plants are exposed to chromium stress (T1) as compared to the control (T0) at 30, 60 and 90 DAS respectively.

Keywords: Biofertilizer, chromium, germination percentage, heavy metal stress, plant height

Introduction
In different agro-climatic conditions maize which is scientifically known as *Zea mays* L. which is one of the most significant widely adaptable crops. Due to its maximum yield which is the response of genetically potential as compared to other cereal crop known as cereal's queen. Around a hundred sixty-five nations, it is developed on very nearly 190 million hectares under the more extensive scope of soil, atmosphere, biodiversity and the executive's practices which subsidizes 39% in world grain creation. The US of America is the biggest producer of maize having 36% of the all-out creation on the planet and principle wellspring of US economy. In India, it is developed consistently however by and large it is *Kharif* crop having 85% region under development in the season. In India maize is third most substantial grain crop among the *Oryza sativa* and *Triticum aestivem*. It subsidizes for about 10 per cent of complete nourishment grain production in the nation. Maize creation is assessed to be 21,810 thousand tons in 2015-16.

Grains of cereals are the products of developed grasses. They furnish mankind with greater sustenance and almost 50% of the all-out caloric necessity than some other nourishment class. Grains crops utilized for nourishment, just maize, rice and wheat are significant human nourishment sources, representing 94% of all grains utilization (FAO. 2012). Utilization of these grains is lower than the genuine figures because of loss of nutrients and minerals as a large portion of the nutrients are amassed in the peripheral grain layer which is expelled in processing process and evacuated wheat or external layers additionally utilized as creature feed (Milazzo, A. 1986 and USAID 2002). In the United States depending on the product 60% to 100% extraction rate for maize varies in most countries whereas the range for yellow maize goods is 60-65%. Rate of extraction for super is 62% whereas for sifted is 79-89% and unsifted is 99% in South Africa. (National Chamber of Milling, South Africa. 2013).

Chromium which is one of the chemical element in group six with steel- grey and a hard transition metal. Due to its anti-corrosive properties, it is mainly used in stainless steel. It is extremely valuable as metal and capable of polish during resisting tarnishing. Chromium is a

Greek word, Chroma known as colour. It is also known for high corrosion resistance and hardness.

Due to the oxidative properties and Ph of that particular area there exist the relation between the trivalent chromium and hexavalent chromium. It ranges used for the oral toxicity for chromium (VI) is 1.50 to 3.29 miligram per kg. Formerly Cr translocated enters body cell chromium (VI) reduced to chromium (III) by several mechanisms. (Dayan *et al.*, 2001) [6]. The carcinogenetic of the chromate ions known for the longest time and in 1890 the chances of cancer were raised in those labourers which worked in the chromate colour company (Newman 1890) [16]. To portray the genotoxicity of chromium (VI) three mechanism have been proposed. The primary one is intensely receptive hydroxyl radicals and other is by the decrease of hexavalent chromium to trivalent chromium through a mechanism which comprises authoritative chromium (V), transported by depletion within the cell where compoundsof chromium (IV) to the DNA. The third components incorporate official to the DNA of the conclusion item of the chromium (III) diminishment (Cohen 1993) [5].

Trichoderma

Trichoderma species extremely instinctively in the root, soil and foliar situations. From a longer period, it is known for various quite while that they make a wide stretch out of against microbial (Sivasithamparam 1998) which they parasitize different parasites. They can additionally rival diverse microbes representation, its view from seeds crucial exudates that sustain the life of plant- pathogenic life forms generally contend with topsoil microbes enhancement through besides space through propagules germination (Howell *et al*, 2002) [7].

Trichoderma species are the foremost transcendent kind of parasites in soils and they appear far and wide in almost all dirt sorts. They are minute and are overall viewed as hurtful plant symbionts. As such, they shape a profitable, or usually helpful, relationship with plants. This beneficial relationship is the thing that biofertilizers endeavour to mishandle. The idea is direct, by including more Trichoderma the plant gets more advantage. The greater part of the Trichoderma species recorded here give the advantages from over. It's basic to take note of that Trichoderma applications can be made as a seed treatment, as a foliar application. Bioremediation with the use of organism known as mycoremediation, plants which are called phytoremediation or with microbes known as bacterial bioremediation.

Rhizobium

More prominent assembling of grains delivers more noteworthy assembling cost and contaminates the soil environmental factors because of unbalanced utilization of synthetic manures. In this manner, crop researchers are investigating a decision supply especially biofertilizers which are cost-effective and environmental factors amicable. Among all other biofertilizers rhizobium is one of the most important biofertilizers which is used almost in all countries. As of late, it is moreover found in a graminaceous family like wheat, barley, rice, maize and different cereals crop rhizobium can make an association with cereals as endophytic without forming the nodule like structure. Expanding the limit of rhizobia as biofertilizer there is bettering activity in non-vegetables exceptionally oat grains would be gainful mechanical expertise for increased harvest yields among asset poor ranchers. Ongoing discoveries affirmed both additional yield bettering and biofertilizer traits in oats crops due to rhizobial immunization. Along these lines, correspondingly query right now be in a situation to help reasonable biofertilizer science for expanded and condition wonderful grain fabricating framework (Mia *et al*., 2010) [15].

Mycorrhizae

About 80% of plant AMF is one of the characterize crew of root obligate biotrophs that have mutual profits. In an opinion that they award the water host, supplements as well as pathogen safety, in change for photosynthetic objects consequently taken into consideration as commonplace biofertilizers. AMF are essential biotic soil components which, while lacking or devastated, can prompt a less effective biological system working. By the way of restoring the characteristic level of AMF can symbolize an authentic decision to traditional treatment rehearses, with the aim of feasible sustainable agriculture. The most significant technologies which can be received to achieve objective which immediate re-presentation of AMF propagules(inoculum) coverts an objective soil. Soil microbes for example arbuscular mycorrhizal growth portray a key connection between vegetation and soil mineral supplements.

In this way, they are hoarding developing movement as a home has grown composts. Having a place with the phylum Glomeromycota it has a place with commit symbionts (Schulter *et al.*, 2001), shape mutualistic associations with adjoin 80% of plant species in the land, together with a few agrarian harvests. They also provide the mineral nutrients as well as the water for the photosynthesis to the host plants (Smith *et al.*,2008) [17]. The AMF mycelium which developed from roots helps to get the nutrient deep from the soil where the roots of the host plant cannot reach (Smith *et al.*, 2000). Moreover, parasitic hyphae are parcels more slender than roots and are subsequently in a situation to enter littler pores (Allen, 2011) [2].

Methodology

The research work was carried out during the *Zaid* season entitled as **"**Trichoderma, Rhizobium and Mycorrhiza Mediated Mitigation of Chromium (VI) Toxicity in Maize (*Zea mays* L.)" at School of Agriculture, Lovely Professional University (LPU), Phagwara, Punjab. The information of the criteria used, the techniques adopted for experimental work, for the evaluation of treatments during the entire season of experimental investigation are mainly described in this chapter.

The pot experiment was conducted in the poly house of the School of Agriculture, Lovely Professional University, Jalandhar, Punjab with one variety of Maize PMH -1. Maize variety was taken from Punjab Agriculture University, Punjab. Pot size for the experiment was diameter: 30 cm and height 25 cm and area of pot was 0.0706 m^2. Chromium stress was created in the plant by exogenous application of Chromium dichromate in soil. One best concentration after initial screening within the range of 1-100 ppm of Cr was finally selected. There is one concentration of heavy metals after the screening that is 100 ppm per pot, was applied in the soil for creating stress in Maize plant. Trichoderma, Rhizobium and Mycorrhiza were applied at the rate of the recommended dose in soil. For Trichoderma recommended dose is 20-25 gm/100m^2 and for 0.0706m2 it is 17.6 mg. For mycorrhiza, the recommended dose is 10 kg/ha and for 0.0706 m2 it is 70 mg. For rhizobium the recommended 3 litres for 1000 kg of farmyard manure and 1 kg of FYM it is

3ml. The various measurements were made at three stages such as 30 day, 60 days and 90 days.

Treatments Details

T0- Control; T1- Chromium (Potassium dichromate:100ppm/pot); T2-Trichoderma (*T.viride*:17.6 mg/pot); T3-Rhizobium (*R. trifoli*: 3ml in 1 kg of FYM/pot); T4-Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot); T5-Chromium (Potassium dichromate:100 ppm/pot) + Trichoderma (*T.viride*:17.6mg/pot); T6- Chromium (Potassium dichromate:100ppm/pot) + Rhizobium (*R. trifoli*: 3ml in 1 kg of FYM/pot); T7- Chromium (Potassium dichromate:100 ppm/pot)+Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot): T8- Chromium (Potassium dichromate:100 ppm/pot) + Trichoderma (*T.viride*:17.6mg/pot) + Rhizobium (*R. trifoli*: 3ml in 1 kg of FYM/pot); T9-Chromium (Potassium dichromate:100 ppm/pot) + Trichoderma (*T.viride*:17.6mg/pot)+ Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot); T10- Chromium (Potassium dichromate:100 ppm/pot)+Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot) + Rhizobium (*R. trifoli*: 3ml in 1 kg of FYM/pot); T11- Chromium (Potassium dichromate:100 ppm/pot)+Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot)+Trichoderma (*T.viride*:19mg/pot) + Rhizobium (*R. trifoli*: 3ml in 1 kg of FYM/pot); T12- Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot)+Trichoderma (*T.viride*:17.6 mg/pot) + Rhizobium (*R. trifoli*: 3ml in 1 kg of FYM/pot).

Observation was Recorded

Germination percentage (%)

Germination percentage was recorded from the total number of seeds sown and the total number of seeds emerged. The experiment followed a completely randomized design with three replicates with 10 seeds per replicate per treatment of Maize. The seeds began to emerge seven days after sowing, and seedling emerge was counted thereafter every day and continued for one week till the 15 days.

Plant Height (cm)

Plant height measured at 30, 60 and 90 days after sowing. The measuring scale was used to measure the height of the plant from the ground to the top surface to the topmost leaf of the plant (Fig. 1).

Fig 1: Plant Height was taken with measuring scale

Results and Discussion

Germination Percentage

Effect of applied biofertilizer on germination was studied in maize variety PMH-1 under the chromium toxicity. After 15 DAS data were taken by counting the plants in each pot and germination percentage was calculated as it was shown in (Table 1, fig 2). The average germination percentage was gradually decreased with 33.32% in treatment where chromium stress was created(T1) as compared to control (T0) where Amna *et al.,* (2015) also reported that the effect of chromium on the growth of maize plants. When biofertilizer i.e mycorrhiza, trichoderma and rhizobium was applied along with chromium (T5)Chromium+Trichoderma, (T6) Chromium + Rhizobium, (T7) Chromium + Mycorrhiza it was found that germination percentage was enhanced with the application of biofertilizer with 41.66%, 46.15% and 46.15% respectively as compared to the chromium (T1). Singh *et al.,* (2015) this study was aimed to investigate that different concentration of chromium and lead in seedlings of maize. From the result it was found that at 300 micromolar concentration reduced the growth of maize like germination percentage, plant height, stem girth, leaves a number and physiological parameters of maize plants. At 200 micromolar concentration of chromium, the shoot and root parts were affected whereas the lead was not too much toxic as that of chromium. It was found that exogenous application of the biofertilizer can mitigate the chromium stress by enhancing the average germination percentage. Ahmad *et al., (*2016) [1] this study was done with the pot experiment to evaluate the effects of Al and Cr on different parameters like morph physiological and different biochemical parameters in plant parts. At pre and post silking stage data were collected when plants were exposed to Al and Cr and plants with was uncontaminated considered as the control. From the result, it was represented that both the metal reduced the maize growth as well as yield by photosynthetic reduction. In the terrestrial plant's mycorrhizae soil fungi able to develop the symbiotic association in which it provides the nutrients and water to the host plant in returns to get the carbon for their survival (Kumar and Dwivedi, 2014, Kumar and Dwivedi, 2018) [13, 10]. From the different research work, it was indicated that mycorrhizae have the benefits effects on the host plant growth under different stresses like drought, salinity, heavy metal stress, etc. Many more have to be clarified some mechanisms which increase the fungal tolerance and host plant mechanism under heavy metal stress (Kumar and Dwivedi 2018 a, b, c, Beshamgan *et al*., 2019) [10-12, 4].

Table 1: Germination (%) of maize during the *Zaid* season

Treatments	Germination (%)
T0	$70.00^{ab}\pm10.00$
T1	$46.67^{b}\pm25.17$
T2	$86.67^{a}\pm15.28$
T3	$60.00^{ab}\pm10.00$
T4	$73.33^{ab}\pm5.77$
T5	$80.00^{ab}\pm26.46$
T6	$86.67^{a}\pm15.28$
T7	$86.67^{a}\pm15.28$
T8	$86.67^{a}\pm5.77$
T9	$86.67^{a}\pm11.55$
T10	$86.67^{a}\pm5.77$
T11	$53.33^{ab}\pm40.41$
T12	$76.67^{ab}\pm11.55$

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$ T0- Control; T1- Chromium (Cr VI); T2-Trichoderma; T3-Rhizobium; T4-Endomycorrhizal fungi (AMF); T5-Chromium (Cr VI) + Trichoderma; T6-Chromium (Cr VI) + Rhizobium; T7- Chromium (CrVI)+Endomycorrhizal fungi (AMF); T8- Chromium (Cr VI) + Trichoderma + Rhizobium; T9-Chromium (Cr VI) + Trichoderma + Endomycorrhizal fungi(AMF); T10-Chromium(Cr VI)+Endomycorrhizal fungi (AMF) + Rhizobium; T11- Chromium (Cr VI)+Endomycorrhizal fungi (AMF)+Trichoderma+Rhizobium; T12-Endomycorrhizal fungi (AMF) +Trichoderma + Rhizobium.

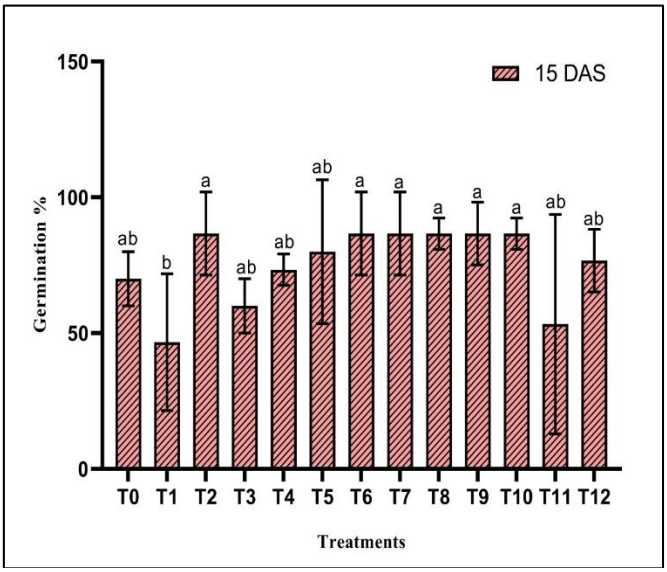

Fig 2: Germination (%) of maize during the *Zaid* season

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Chromium (Cr VI); T2-Trichoderma; T3-Rhizobium; T4-Endomycorrhizal fungi (AMF); T5-Chromium (Cr VI) + Trichoderma; T6-Chromium (Cr VI) + Rhizobium; T7- Chromium (CrVI)+Endomycorrhizal fungi (AMF); T8- Chromium (Cr VI) + Trichoderma + Rhizobium; T9-Chromium (Cr VI) + Trichoderma + Endomycorrhizal fungi(AMF); T10-Chromium(Cr VI)+Endomycorrhizal fungi (AMF) + Rhizobium; T11- Chromium (Cr VI)+Endomycorrhizal fungi (AMF)+Trichoderma+Rhizobium; T12-Endomycorrhizal fungi (AMF) +Trichoderma + Rhizobium.

Plant height (cm)

Mitigation effect of the Trichoderma, Rhizobium and Mycorrhiza and their combined application on plant height (cm) was evaluated in maize variety PMH-1 during the *Zaid* season in the year of 2019 grown under the chromium metal stress. Data were recorded at different growth interval 30, 60 and 90 DAS shown in (Table 2, Fig. 3). Average plant height was significantly decreased by 23.29%, 14.28% and 18.65% when plants are exposed to chromium stress (T1) as compared to the control (T0) at 30, 60 and 90 DAS respectively. Similarly, Anjum *et al.*, (2016) study were conducted to know about the effect of varying chromium toxicity in the maize plants in which level of stress of Cr affect the growth of plants, photosynthesis, different responses of yield and capacities of gas exchange into two-hybrid of maize i.e Wan Dan 13 and Run Nong 35 were grown in different concentration of contaminated soil in pots. It was also found that the height of the plant, leaves number, area of leaves, a diameter of the stem, dry weight and fresh weight also reduced and reduction of yield attributes was also reduced. Exogenous application of endomycorrhiza in the soil (T4) average plant height was decreased by 10.08%, 15.2% at 30 and 60 DAS respectively as compared to the chromium stress (T0) but at 90 DAS the average plant height was increased by 25.74% in mycorrhiza treatment (T4) as compared to the chromium stress (T0). Lenoir *et al.*, (2016) suggested that there are some fungal species due to some special morphological as well as the physiological potential of fungal species having the fast growth to produce the spores. In the contaminated soil, the fungal can prevent the plants by absorbing the heavy metal as well as by restricting the interaction of host plant with heavy metals. The combined application of rhizobium and mycorrhiza along with the chromium metal (T10) it was observed that average plant height was significantly increased with 9.35%, 28.21% and 9.83% as compared to the treatment where trichoderma with mycorrhiza was applied in contaminated soil with chromium (T9) at 30, 60 and 90 DAS. The application of mycorrhiza showed the best result with a combined application of rhizobium then that of trichoderma. Kumar P. (2018b) [11] conducted a pot experiment, in which mycorrhiza and putrescine were applied as the ameliorative agents for Cd toxicity. The combination of mycorrhiza and putrescine showed a better result for the mitigation of Cd casing toxicity in plant height and leaf number per plant.

Table 2: Plant height (cm) of maize during the *Zaid* season

Treatments	30 DAS	60DAS	90 DAS
T0	21.46^{a}± 0.55	45.23^{a}± 1.30	64.33^{a}±3.06
T1	16.46^{cde}±2.15	38.77^{h}±0.96	52.33^{bc}±2.52
T2	15.56^{cde} ±3.72	37.97^{dh}±1.29	55.67^{b}±4.16
T3	14.20^{def}±0.75	34.50^{f}±0.56	49.33^{cd}±1.53
T4	14.80^{def} ±1.08	32.87^{g} ±0.71	65.80^{a}±4.23
T5	17.96^{bc}±1.76	33.83^{g}±0.29	44.33^{de}±4.16
T6	16.76^{cd}±1.50	32.43^{g}±0.55	44.67^{de}±3.51
T7	15.80^{cde} ±0.26	37.20^{de}±0.70	44.33^{de}±3.51
T8	13.80^{ef}±0.75	32.93^{g}±0.31	30.00^{g}±1.00
T9	15.60^{cde} ±0.60	30.13^{h}±0.71	37.33^{f}±2.08
T10	17.06^{cd}±0.15	38.63^{dh}±0.55	41.00^{ef}±1.00
T11	12.60^{f}±1.15	36.13^{e}±0.32	37.00^{f}±2.65
T12	20.03^{ab}±1.00	40.40^{b}±1.57	40.00^{ef}±2.00

where DAS: Days after sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Chromium (Cr VI); T2-Trichoderma; T3-Rhizobium; T4-Endomycorrhizal fungi (AMF); T5-Chromium (Cr VI) + Trichoderma; T6-Chromium (Cr VI) + Rhizobium; T7- Chromium (CrVI)+Endomycorrhizal fungi (AMF); T8- Chromium (Cr VI) + Trichoderma + Rhizobium; T9-Chromium (Cr VI) + Trichoderma + Endomycorrhizal fungi(AMF); T10-Chromium(Cr VI)+Endomycorrhizal fungi (AMF) + Rhizobium; T11- Chromium (Cr VI)+Endomycorrhizal fungi (AMF)+Trichoderma+Rhizobium; T12-Endomycorrhizal fungi (AMF) +Trichoderma + Rhizobium.

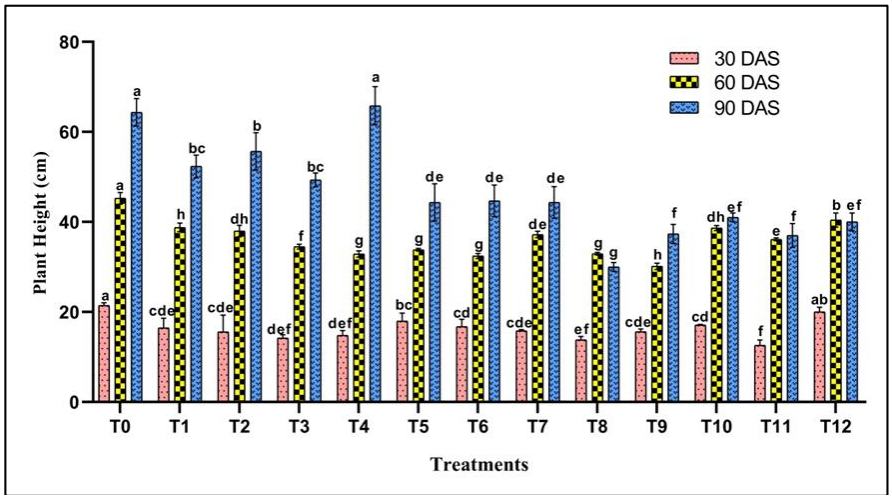

Fig 3: Plant height (cm) of maize during the *Zaid* season

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Chromium (Cr VI); T2- Trichoderma; T3-Rhizobium; T4-Endomycorrhizal fungi (AMF); T5-Chromium (CrVI) + Trichoderma; T6- Chromium (Cr VI) + Rhizobium; T7- Chromium (CrVI)+Endomycorrhizal fungi (AMF); T8- Chromium (Cr VI) + Trichoderma + Rhizobium; T9-Chromium (Cr VI) + Trichoderma + Endomycorrhizal fungi(AMF); T10- Chromium(Cr VI)+Endomycorrhizal fungi (AMF) + Rhizobium; T11- Chromium (Cr VI)+Endomycorrhizal fungi (AMF)+Trichoderma+Rhizobium; T12-Endomycorrhizal fungi (AMF) +Trichoderma + Rhizobium

Conclusion

From the result, it was concluded that the germination percentage was reduced when exposed to chromium toxicity as compared to control. Exogenous application of biofertilizers with chromium metal was able to mitigate the chromium toxicity by enhancing the germination percentage as compared to chromium stress. Similarly the combined application of Mycorrhiza and trichoderma along with chromium metal able to reduce the toxicity of chromium by improving the plant height as compared to the chromium stress. It was also found that the combined application biofertilizers were more effective in mitigating the toxicity stress of Cr contaminated soil by improving the potential production of maize**.**

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Author Contributions

P.K. designed the study, established the biochemical protocols, P.D performed the experiments and collected the data analyzed and interpreted the data. P.K. wrote the paper.

Conflict of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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