ORIGINAL PAPER



Management of chromium(VI)-contaminated soils through synergistic application of vermicompost, chromate reducing rhizobacteria and Arbuscular mycorrhizal fungi (AMF) reduced plant toxicity and improved yield attributes in *Ocimum basilicum* L.

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Abstract

An integrated approach involving vermicompost, chromate reducing bacteria and AMF was tested to manage the toxic impacts of Cr(VI) on *Ocimum basilicum* as a model plant. Pot experiments were conducted on *O. basilicum* plants in an artificially Cr(VI)-contaminated soil in two phases of experiment as bioinoculants experiment and vermicompost experiment. In the first phase of the bioinoculants experiment the series of gradient concentrations of Cr(VI) (0, 25, 50 and 100 mg kg⁻¹ in soil) were evaluated with previously isolated four efficient Cr(VI)-reducing rhizo-bacterial strains (*Bacillus Cereus* strain SUCR 44, BC; *Microbacterium* sp. strain SUCR 140, MB; *Bacillus thuringiensis* strain SUCR186, BT; and *Bacillus subtilis* strain SUCR188; BS) along with Arbuscular Mycorrhizal Fungus—*Glomus fasciculatum* (GF) in alone and in co-inoculation form. In the second experiment (vermicompost) the best performing strain (MB) was tested alone or in combination with GF along with different doses of vermicompost. It was observed that vermicompost by itself could be useful in decreasing the bioavailable Cr(VI), uptake of Cr besides improving the nutritional status of plants. The vermicompost also played an important and indirect role and improved herb yield by supporting the multiplication of MB (*Microbacterium* sp.), an efficient chromate reducing rhizobacteria, that further decreased the bioavailable and toxic form of Cr and improved population and colonization of GF too. The translocation of Cr(VI) was averted through improved colonization of GF, also prevented higher accumulation of Cr in aerial parts (leafy herb) of *O. basilicum*.

Keywords Vermicompost \cdot Glomus fasciculatum \cdot Cr(VI) \cdot Cr(VI) reducing bacteria \cdot Bioavailability

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Introduction

The growing population and their demands for products are ever-increasing. To have a sustainable supply chain, countries are running for rapid industrialization. Various industries involves chromium Cr(VI) [Cr(VI)] uses in several industrial processes such as—in tanning, electroplating, ceramics, dyes, paints and pigments manufacturing, textile processing, metal finishing, wood processing and photographic sensitizer manufacturing units. The byproducts and effluents from these industries are serious threat to the environment and living being. In aqueous phase Cr(VI) bears structural similarity with SO_4^{2-} , entered into cells via sulfate transport pathways (Patra et al. 2010), rapidly reduced generates free radical (Mabbett and Macaskie 2001). Due to this, it is noxious (Wise et al. 2004), mutagenic (Puzon et al. 2002), carcinogenic (Codd et al. 2003), and teratogenic (Asmatullah et al. 1998) besides causing DNA break (Mabbett and Macaskie 2001) and altered gene expression (Bagchi et al. 2002). Due to this, it is renowned as a precedence pollutant (Cheung and Gu 2007). The adverse health effects associated with Cr(VI) exposure includes respiratory cancer, asthma, skin, nose and eye irritation, chrome ulcers, perforated eardrums, kidney and liver damage, pulmonary congestion, erosion and discoloration of the teeth (USEPA 1998). The median lethal dose of Cr(VI) is 50–150 mg/kg (Katz and Salem 1992). The World Health Organization recommends a maximum allowable concentration of 50 µg/L of Cr(VI) in drinking water (WHO 1996). Although Cr(VI) is highly toxic but its trivalent form, i.e., Cr(III) is an essential micronutrient for animal and human being, involved in protein and glucose metabolism (Vincent 2000), enzyme stimulation (Karuppanapandian et al. 2009), nucleic acid stabilization (Snow and Xu 1991) and is relatively inert and much less toxic (Krishna and Philip 2005).

In underdeveloped and developing countries, the lenient regulations on effluents and discharges from these industries are of great concern (Patra et al. 2010) and even posing serious threats to agricultural soils (Gupta et al. 2019) if discharged in surrounding to it. The continuous discharge and uses of contaminated water or source for irrigation effected by it (Jiménez 2006; Abaidoo et al. 2010) further deteriorates the situation leading to heavy crop yield losses. Once the Cr(VI) entered to the plant system, it gets magnified inside the plant and its products remains unfit to be used due to the concentrations crossing the permissive limits fit to be used (Vernay et al. 2007; Soni et al. 2014a, b; Rai et al. 2002; Oliveira 2012). Excessive Cr(VI) accumulation itself, causes severe toxicity to plants leading to altered metabolic processes, impaired photosynthesis, hampered nutrients uptake, inactivation of mitochondrial electron transport, chlorosis, and membrane damage resulting in reduced root growth, stunting and finally leading to plants death (Soni et al. 2014a, b; Shanker et al. 2005).

Globally a large population (80%) still relay on herbal products, supplements for their primary healthcare and immune boosting (Ekor 2014; Soni et al. 2022). In the time of COVID-19 pandemic, demands and usage of herbal supplements and drugs are ever-increasing (Soni et al 2021, 2022). Particularly in Indian subcontinent, masses still relay on traditional system of medicine obtained directly from medicinal plants for maintenance of their day-to-day life and immune boosting (Soni et al. 2021). The medicinal plants contain high economical value secondary metabolites which either directly or indirectly used as raw materials for pharmaceutical, cosmetic and perfumery industries (Tiwari et al. 2017, 2021). Therefore, there is a continuous surge in the demands of raw materials and herbs and herbal products. It is evident that global warming and climate change have impacted on land and water availability for humans and agriculture. In the present scenario safe water management is almost challenging issue, and it is highly recommended to reuse water for agricultural purposes (Abaidoo et al. 2010). Farmers with no option left, when uses such water for irrigation (Jiménez 2006) or either contaminated sites for cultivation severely impacts plant growth/yield and economy. Not only this if the plants or products from affected areas are used they raise safety issues due to magnified accumulated metals (Zheljazkov and Nielsen 1996a, b). The safety concerns of medicinal herbs-based drugs adulterated with metals beyond permissible limits are not new (Rai et al. 2001; Saper et al. 2008; Dargan et al. 2008; Breeher et al. 2013) and attention has been raised from time to time. Looking from the point of remediation many researchers tried aromatic plants as a remedial solution which has some positive aspects, because of their by-products basically essential oils, remains safe from adulteration of Cr(VI) (Gupta et al. 2013). Therefore, they can be a wise option to look into as a part of integrated management system for Cr(VI) remediation in contaminated sites with usable products obtained without adverse losses (Gupta et al. 2013).

This is well established that plant rhizospheric zone has enriched beneficial microbes which can positively interact with root system and forms first line of defences against both biotic (phytopathogens) and abiotic stresses (Tiwari et al 2017). They not act antagonist microbes but also help in mitigating abiotic stresses such as detoxifying metal stress or hazardous compounds present in the vicinity (Burd et al. 2000; Rajkumar and Freitas 2008). The Cr(VI) stress management thus can be achieved via enriching Cr(VI) tolerant microbes (Soni et al. 2014a, b; Wani et al. 2009) which might have other plant beneficial properties such as-plant growth promoting (PGP) potentials, such as nitrogen fixation, phosphate solubilization, siderophore and growth hormones production (Gupta et al. 2019; Glick et al. 1999; Khan and Zaidi 2007). These microbes adapt either enzymatic strategy or manage metal stress via production of metabolites (Losi and Frankenberger 1994), or even may be via accumulation and sequestration (Mamaril et al. 1997). Therefore, isolation and exploration of such microbes have coupled with broad range of potentials, i.e., PGP, or soil remediation will be a better safe management strategy for sustainable agro-technologies under heavy metal stress (Rajkumar et al. 2005, 2006).

The beneficial role of mycorrhiza spans around enhancing plant health, nutrition, quality by stimulating the synthesis of secondary metabolites/bioactive compounds (Singh et al. 2013a, b, c; Tiwari et al. 2017). They also play important role in tolerance to abiotic (Vivas et al. 2006) and biotic stresses (Saikia et al. 2013; Gupta et al. 2014), or even enhance antioxidant activities (Gianinazzi 2008, 2010). Their co-inoculation with beneficial bacterium further improves plant growth, establishment, stress tolerance and mitigation of heavy metals under metal-contaminated conditions (Vivas et al. 2003, 2005, 2006) including Cr stress mitigation too (Soni et al. 2014a). Mycorrhiza has been regarded to help in mitigating metal stress by immobilizing the metals or sorting the metals and thus lowering the toxicity of metal toxicity in plants (Gother and Paszkowski 2006).

Vermicompost is a finely divided peat-like material mainly originates from organic wastes. They when mixed with soil provides better porosity, aeration, drainage and have provides good moisture content because of its high capacity of water holding (Edwards and Burrows 1988). They are highly recommended for the growers and farmers as they stimulate plant growth and development and provides better coping with abiotic stresses to the plants. (Pande et al. 2007; Jadia and Fulekar 2008; Koka et al. 2019). Even it is evident from the research (Koz'uh et al. 2000) that humic acid and fulvic acid present in vermicompost have potentials to reduce Cr(VI) stress in plants (Chaab et al. 2016). Experimental evidence from our previous studies also revealed that vermicompost acts as a proliferating substrate for the applied microbes and enhances growth and development (Kalra et al. 2010; Singh et al. 2012a, 2013b).

Sweet basil (Ocimum basilicum L. (family Lamiaceae), is an annual herb and considered as an important medicinal and aromatic plant due to its marvelous beneficial potentials (Grayer et al. 1996; Tiwari et al. 2017). They have been traditionally used in many kinds of herbal drug preparations, supplementary treatments for stresses, aches, inflammations, asthma and diabetes (Telci et al. 2006). Their leaves contain an essential oil used as flavorings agents in foods and beverages, as fragrances, as fungicides, or insecticides and in various pharmaceutical and industrial products (Singh et al. 2013b; Tiwari et al. 2017, 2021). Because of its high economical value and industrial importance, sweet basil is highly acclaimed commercial crop in many countries, including Indian subcontinent (Padalia et al. 2017). With the multifaceted potentials and aromatic nature of the crop we selected this as a model plant for Cr (VI) stress studies. We designed the experiments to evaluate them under Cr (VI) stress and their impacts on yield attributes. Furthermore, we looked for an integrated method of management towards minimizing economical losses due to Cr (VI) stress. Considering the Cr reducing and chelating properties of vermicompost, we undertook this study to establish the integrated potentials of vermicompost along with MB and GF in synergizing the chromate reduction activity and Cr toxicity in plants. We also were interested to look into, if GF could participate in improved mycorrhization and nutrient uptake. We hypothesize that microbe based remediation of metal stress will provide safe and wiser stress management strategies which can be employed for future agro-technologies. This study further will pave new avenues for search of efficient microorganisms that can be employed for metal stress mitigation in different crops and plants without much impacting the product. The outcomes from this study will generate a consortium for better management of Cr (VI) stress in *in-planta*, as well as in soils too. Furthermore, it will contribute to higher yields thus lowering the economical losses in *O. basilicum* when cultivated under Cr (VI) stress.

Materials and methods

Preparation of soil samples with artificial Cr(VI) contamination

A set of experiments were conducted which were divided into two phases-phase 1-bioinoculants experiment, where we screened different microbial treatments in single and in combination for their potentials of Cr(VI) stress management and plant growth promotion. While the second phase of experiment (vermicompost experiment) dealt with integrated approach involving vermicompost as a substrate having selfpotentials for Cr(VI) stress reduction, as well as supporting proliferation of microbes and indirectly contributing in the Cr(VI) management and toxicity reduction. The artificial contamination of soil with Cr(VI) was performed by following the previously described method (Papassiopi et al. 2009). In the bioinoculants experiment, respective concentrations of artificially Cr(VI)- (25 mg kg⁻¹, 50 mg kg⁻¹, and 100 mg kg^{-1}) contaminated soil (autoclaved) were prepared for the pot experiment. In the second experiment (trial with vermicompost), the most effective known concentration of Cr(VI) (100 mg kg⁻¹ soil) was mixed in soil containing different doses of sterilized vermicompost (0 t ha⁻¹, 2.5 t ha⁻¹, 5 t ha^{-1} , and 10 t ha^{-1}).

The left over de-oiled herb distillation waste from aromatic plants named such as *Mentha arvensis*, *Cymbopogon flexuosus* and *C. winterianus* were used for production of vermicompost. The vermicompost was produced at the vermicomposting unit housed at Microbial Technology Department of CSIR–CIMAP, Lucknow, U.P India, using earthworm, *Eudrilius eugineae*. The leftover plant herb mixture was finely chopped and fed to the vermicomposting unit. The unit was regularly monitored for proper moisture content for 90 days to achieve proper degradation and conversion into vermicompost by *Eudrilius eugineae* (Kalra et al. 2010; Singh et al. 2012c, 2013b). The vermicompost thus produced was analyzed for constituents and contained 1.10% N, 0.62% P and 0.76% K.

Plant material and growth conditions

The experiments were performed under the controlled glasshouse conditions with optimum temperature range $(30-40^{\circ}C)$, moisture content $(60-70^{\circ})$, and photoperiod

[Light (14 h)/Dark (10 h)]. The sandy loam soil (Ustifluvent) was used for this experiment having following characteristics [pH 7.26, EC 0.42 dSm⁻¹, organic carbon 3.37 g kg⁻¹, available N (alkaline permanganate extractable) 183 kg ha⁻¹, available P (0.50 M NaHCO₃ extractable) 16.3 kg ha⁻¹, and available K (1 N NH₄OAc extractable) 94 kg ha⁻¹].

Preparation of bio-inoculums

Cr(VI) reducing bacterial inoculums

The bacterial culture and their vermicompost based inoculum were produced as per the protocols described earlier (Soni et al. 2014a, b). At the time of application, the population of bacterial strains was maintained at $2.0-2.4 \times 10^9$ CFU g⁻¹/vermicompost. A five gram of such vermicompost based inoculum was applied to each pot by placing near to the seedlings rhizospheric vicinity.

Glomus fasciculatum (GF) inoculum

The inoculum of *Glomus fasciculatum* (GF) species was obtained from repository of Microbial Culture Collection housed at CSIR–CIMAP, Lucknow, India. The propagation and application of GF was done by following the methods as described by Singh et al. (2012b, 2013b). At a time of application to the pot experiments, the inoculum concentration was adjusted to 7.0 ± 0.5 spores g⁻¹.

Seed treatment

Seeds of *O. basilicum* cv. CIM-Saumya were procured form CSIR–CIMAP farm center (Lucknow, India), were sorted for healthy looking ones. They were surface sterilized with application of sodium hypo-chloride (1%) for 5 min followed by 3 times washing with sterilized deionized water. The seeds were transferred to the earthen pan containing soil—vermicompost mixture (both autoclaved, 10:1 v/v) and placed under glasshouse conditions as described previously under dark conditions till germination. Furthermore, the germinated seeds were allowed to raise to 2 to 3 leaf staged seedlings. The seedlings were used for pot experiments under glasshouse conditions as mentioned before.

Experimental design

The experiment was set up in a completely randomized design (CRD) with three replications per treatment (Table 1). Two seedlings were transplanted in each pot (10 inch diameter containing 8.5 kg sterilized potting mixture) and allowed to get established. After 1 week, pots were homogenized by removing the extra one plant and leaving behind one plant per pot. The optimum moisture level in soil was maintained

Table	Table 1 Experimental design and microbial treatments of the pot experiments conducted in controlled conditions	s of the pot experim	ents conducted in contr	olled conditions	
S. no.	S. no. Treatments	Abbreviation used Description	Description	Bioinoculants experiment	Vermicompost experiment
	*Control	CN	Uninoculated	Gradient concentration of Cr(VI) (0, 25, 50	Gradient doses of vermicompost (0 t ha ⁻¹ ,
7	*Glomus fasciculatum	Gf	Gf inoculated	and 100 mg kg ⁻¹ in soil) were taken	2.5 t ha^{-1} , 5 t ha^{-1} , 10 t ha^{-1}) were taken at
ю	Bacillus cereus strain SUCR44)	BC	BC inoculated		100 mg kg ⁻¹ Cr(VI) in potting mixture
4	*Microbacterium sp. strain SUCR140	MB	MB inoculated		
5	Bacillus thuringiensis strain SUCR186	BT	BT inoculated		
9	Bacillus subtilis strain SUCR188	BS	BS inoculated		
٢	Bacillus cereus strain SUCR44 + Glomus fasciculatum	BC+GF	BC+GF inoculated		
8	* <i>Microbacterium</i> sp. strain SUCR140+ <i>Glo</i> - MB+GF mus fasciculatum	MB+GF	MB + GF inoculated		
6	Bacillus thuringiensis strain SUCR186+Glo- BT+GF mus fasciculatum	BT+GF	BT+GF inoculated		
10	Bacillus subtilis strain SUCR188+Glomus fasciculatum	BS+GF	BS+GF inoculated		
*Treat	*Treatments were selected for Experiment-II				

by application of sterilized water at regular intervals. The experiments were repeated twice at different time intervals under similar set of glasshouse conditions as described previously with 3 biological replications per treatment.

Harvesting and biomass measurements

After completion of 75 days from sowing of seeds the harvesting of the plants were manually performed using sickle. The plants were separated from root and scaled using measuring balance. Th roots were properly washed to remove adherent Cr(VI) on surface of the roots. The length of both root and shoot were measured using meter scale. Biomass (dry weight) was estimated separately for root and shoots from oven dried (at 70 °C till a constant weight was obtained) samples. At a time of harvesting, the soil samples were also collected and preserved at 4 °C till the analysis of microbial populations was conducted.

Determination of bioavailable Cr(VI) [Soluble Cr(VI)] in soil

Bioavailable Cr(VI) in the soil was measured by following the methods of Rtidel and Terytze (1999). A cocktail of soil (10 g), phosphate buffer (48 ml, 0.1 M, pH 8.0), $Al_2(SO4)_3$ (1 ml, 0.4 M) and Na_2SO_3 (1 ml, 1 M) was prepared and shaked (30 min at 250 rpm). Afterwards, 10 mL filtrate (using 0.45 µm filter) was taken and mixed with DDW (20 ml) and NaOCl (1 ml) followed by NaCl (5 g) and H_3PO_4 (1 ml, 7 M) were added. The whole solution was then transferred in a 50 ml volumetric flask. Furthermore, 1 mL of diphenylcarbazide (DPCZ) solution was added and the flask was filled up to the mark with water. After 10 min of incubation the absorbance was recorded at 540 nm. A separate 10 ml of soil filtrate treated in the same manner with only 1 ml of acetone instead of DPCZ solution was used as blank.

Estimation of Cr in plants

The total Cr concentration in root and shoot samples was determined using atomic absorption spectroscopy (Perkin Elmer), using a method previously described by Soni et al. (2014a, b). The pulverized plant samples (200 mg) from each treatment were taken in Teflon tubes containing 10 ml of digestion mixture [Concentrated HNO₃ and HF (2:1, v/v)]. The samples were digested using microdigester (Analytik Jena, Germany) at 200 °C and 200 bar pressure for 75 min (Soni et al. 2014a, b). After digestion, the samples were allowed to cool and filtered through Whatmann (no. 1) filter in a 25 ml of measuring conical flask and the volume of filtrate was made to 25 ml using deionized water. Total Cr content of the digested samples was determined by AAS (Perkin Elmer). Cr uptake in each plant parts (root and shoot) was calculated by bioaccumulation factor (BAF) formula (Ghosh and Singh 2005).

$$BAF = \frac{\text{mg Cr/g of drymass plant}}{\text{mg Cr/Kg of soil}} \times 100$$

The translocation efficiency, i.e., ratio of Cr in shoot and root tissue was calculated by translocation factor (TF) formula (Tappero et al. 2007).

$$TF = \frac{\text{mg Cr/g of drymass shoot}}{\text{mg Cr/g of drymass root}}$$

Microbial population and NPK estimation

The abundance of Gf population in *Ocimum* roots were recorded by following a method described by McGonigle et al. (1990). Wet sieving and a decanting method were used for isolation and estimation of AM fungal spores from soil (Bharti et al. 2013). The populations of bacterial strains were determined using a previously described method by Soni et al. (2014a, b). The serial dilution method was used for estimation of microbial population of the soil samples. The samples were serially diluted in 0.85% saline. 100 µl of such diluted sample were taken and spread on nutrient agar culture plates [supplemented with 100 mg l⁻¹ of Cr(VI)]. Thereafter, plates were incubated at 28 °C. For population estimation, the bacterial colonies developed on culture plates were counted using colony counter (Himedia India). The analysis of uptake of NPK in plant samples was performed according to the procedure of Jackson (1973).

Statistical analysis

The statistical analysis of collected data was performed by analysis of variance (ANOVA), assess through β -version of software ASSISTAT 7.6 (http://www.assistat.com). The significant differences among treatments were based on the *F* test in ANOVA, and means were calculated using Duncan's multiple range test (DMRT) with a significance level of $P \le 0.05$ and $P \le 0.01$. The standard error mean (SEM) in vertical bar chart was computed using Sigma plot 10 (systat software, Inc.).

Results

Effects on plant growth and yield

Bioinoculants experiment

The plants treated with a gradient concentration of Cr(VI) showed an inverse relationship with plant growth

parameters. The toxic impacts of Cr(VI) were observed among all the treatments: however, in comparison to control, the bioinoculants treated showed improvements in growth yield attributes (Tables S1-S9, supplementary files). The maximum reduction in plant growth was observed in plants grown in the soil supplemented with 100 mg kg⁻¹ of Cr(VI) concentration. This concentration of Cr(VI) (100 mg kg⁻¹ of soil) was found to be highly toxic to the plants as the length of the roots, plant height, dry root and shoot biomass were significantly reduced (44.44%, 56.57%, 48.21% and 44.71%, respectively) as compared to the plants not treated with Cr (Fig. 1). However, the application of chromate resistant and reducing strains showed a significant (at $P \le 0.05$) improvement in plant growth parameters; maximum beneficial impacts were found with MB. These effects were more pronounced when these strains were co-inoculated with GF. The coinoculation of MB + GF showed maximum increase in plant growth and yields. As compared to control, coinoculation of MB + GF showed 105%, 127%, 79% and 78% increase in root length, plant height, and dry root and shoot biomass, respectively, at 100 mg kg⁻¹ concentration of Cr(VI). In this regard the potentials of MB (*Microbacterium* sp.) alone and in combination with GF showed a promising response in reducing chromate induced damage although even under severe Cr(VI) stress (100 mg kg⁻¹). Thus, we selected these combinations of treatments for further evaluation under integrated approach of Cr(VI) stress management with vermicompost supplementation (vermicompost experiment—Phase 2).

Vermicompost experiment

The effect of MB based on its performance in the phase 1-bioinoculants experiment (detailed results are included in Tables S1–S9, supplementary files), was selected and





Fig. 1 Effect of bioinoculants on (**A**) root length, (**B**) plant height, (**C**) dry root biomass, and (**D**) dry herb biomass of *Ocimum basilicum* in graded level of Cr(VI) amended soil (0, 25, 50, 100 mg/kg). Error

bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \le 0.05$

evaluated alone and in combination with GF in the pots supplemented with gradient concentration of vermicompost. It was found that with increase the dose of vermicompost the toxicity of Cr(VI) was mitigated thus supported growth and yield of plants (Tables S10-S18, supplementary files). The maximal increment was observed when supplemented with 10 t ha⁻¹ vermicompost. The treatment improved length of the roots, plant height, dry root and shoot biomass increased by 48%, 52%, 47%, and 41%, respectively, as compared to the plants not treated with vermicompost. The co-inoculation of MB + GF in presence of 10 t ha^{-1} of vermicompost have much better mitigation of toxic effect of Cr(VI) than compared to vermicompost alone. The co-inoculation along with vermicompost improved the root length (116%), plant height (92%), dry root biomass (133%) and dry shoot biomass (96%) of O. basilicum as compared to the plants not treated with either microbes as well as vermicompost alone (Fig. 2).

Effect on Cr bioavailability in soil

Bioinoculants experiment

At time of harvesting the bioavailable Cr(VI) was about 37 mg kg⁻¹ (at 100 mg kg⁻¹) in the control pots of both phases of experiments (bioinoculants and vermicompost) (Fig. 3a, b). We found that the inoculation of bacterial strains sufficiently reduced bioavailable Cr(VI) in soil, whereas no such effects were noticed in pots inoculated with GF only. It was also found that no further reduction was observed in bioavailable Cr when bacterial strains and GF were co-inoculated. Considering the control value as 100%, the bioavailability of Cr was reduced by 24% and 28% with the treatments of MB and MB + GF co-inoculations, respectively; although statistically at par with each other.



Fig. 2 Effect of bioinoculants on (A) root length, (B) plant height, (C) dry root biomass, and (D) dry herb biomass of *Ocimum basilicum* in Cr(VI) amended soil (100 mg kg⁻¹) with graded level of

vermicompost doses (0, 2.5, 5.0 and 10 ton/ha). Error bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \le 0.05$

Fig. 3 A Effect of bioinoculants on Cr(VI) bioavailability of *Ocimum basilicum* in graded level of Cr(VI) amended soil (0, 25, 50, 100 mg/kg). **B** Effect of bioinoculants on Cr(VI) bioavailability of *Ocimum basilicum* in Cr(VI) amended soil (100 mg kg⁻¹) with graded level of vermicompost doses (0, 2.5, 5.0 and 10 ton/ha)



Vermicompost experiment

The consistent reduction of bioavailable Cr(VI) was observed with increased vermicompost doses and maximum reduction was noticed at highest doses of vermicompost application (10 t ha⁻¹). We found a 34% higher reduction in bioavailable Cr(VI) was observed when compared to the pots untreated with vermicompost.. The inoculation of MB alone in co-inoculation further showed in reduction of bioavailable Cr(VI). As compare to the control, we found 75% and 76% higher Cr reduction in the treatments of MB and MB + GF, respectively; however, no significant effects of GF alone or in combination with MB were noticed on reduction in bioavailable Cr (Fig. 3b).

Effect on Cr uptake in Ocimum basilicum

Bioinoculants experiment

The uptake of Cr was significantly higher in control plants as compared to that of the plants treated (bacterial inoculums) ones. We observed that as compared to control, the maximum reduction in Cr uptake in roots were observed in plants inoculated with MB alone or in combination with GF; although presence of GF did not made any significant differences in uptake of Cr(VI) in plant roots. While we found that the Cr(VI) uptake was significantly lower in shoots when inoculated together with MB (Fig. 4a, b). Considering the uptake of Cr in control as 100%, the uptake of Cr in roots at 100 mg kg⁻¹ of Cr(VI) was decreased almost by 42% and 44% in the corresponding treatments of MB and MB + GF. Likewise, 37% and 58% decrease were found in Cr uptake by aerial part of plants in aforesaid treatment was observed, respectively (Fig. 4a, b).

Vermicompost experiment

In vermicompost experiment, we found that with increased gradient of vermicompost the Cr uptake by plant roots and shoot system were proportionally decreased. It was observed that maximum impact was seen at the highest doses (10 t ha⁻¹) of vermicompost (31% and 33%, respectively). Furthermore, we observed 27% decreased Cr uptake in the plants inoculated with MB + GF treatment. Likewise, we also found that there was 73% lower Cr uptake in the aerial part of plants of the same mentioned treatment (Fig. 4c, d).





Fig. 4 Effect of bioinoculants on (A) uptake of Cr by root and (B) uptake of Cr by shoot of *Ocimum basilicum* in graded level of Cr(VI) amended soil (0, 25, 50, 100 mg/kg). Effect of bioinoculants on (C) uptake of Cr by root and (D) uptake of Cr by shoot of *Ocimum*

basilicum in Cr(VI) amended soil (100 mg kg⁻¹) with graded level of vermicompost doses (0, 2.5, 5.0 and 10 ton/ha). Error bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \le 0.05$

Fig. 5 A Effect of bioinoculants on bioaccumulation factor in graded level of Cr(VI) amended soil (0, 25, 50, 100 mg/kg). **B** Effect of bioinoculants on bioaccumulation factor in Cr(VI)amended soil (100 mg kg⁻¹) with graded level of vermicompost doses (0, 2.5, 5.0 and 10 ton/ha)



Effect on BAF

Bioinoculants experiment

by 45% and 42% in roots, respectively, at 100 mg kg⁻¹ of Cr(VI) (Fig. 5a).

The data, related to BAF for root and shoot, are presented in Fig. 5. The minimum bioaccumulation of Cr was observed in MB and MB + GF treated plants, although both at par statistically. Considering the control value as 100%, the Cr accumulation by MB + GF and MB was found to be reduced

Vermicompost experiment

The inverse relationship between BAF and vermicompost doses was noticed. From vermicompost Experiment, it was observed that, at highest doses of vermicompost there was 32% less Cr accumulation observed as compared to the plants not treated with vermicompost. Co-inoculation of MB with Gf considerably decreased (78%) the Cr accumulation in plants (Fig. 5b).

Effect on translocation factor

Bioinoculants experiment

The TF values < 1, suggested restricted transport of Cr from root to shoot. At 25 mg kg⁻¹ of Cr(VI) supplementation, a

Fig. 6 A Effect of bioinoculants on translocation factor in graded level of Cr(VI) amended soil (0, 25, 50, 100 mg/kg). **B** Effect of bioinoculants on translocation factor in Cr(VI) amended soil (100 mg kg⁻¹) with graded level of vermicompost doses (0, 2.5, 5.0 and 10 ton/ha)

significant decrease in Cr transportation was observed to the plants inoculated either alone with MB or in co-inoculated form (MB + GF). Further augment of Cr(VI) concentration in soil, had no significant decrease in transportation efficiency among all treatment except in MB + GF inoculated plants. We observed that at higher concentration both MB and GF alone are not effective in restricting the translocation, but the plants treated in co-inoculated form showed relatively lower transport of Cr to the aerial parts. As



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compared to control, a 29% reduced translocation of Cr from root to aerial parts were found in MB + GF treated plants (100 mg kg⁻¹ of Cr(VI) (Fig. 6a).

Vermicompost experiment

The transportation of Cr from root to shoot was also not impacted by MB and GF treatments alone in respite of vermicompost doses. However, we found a reduced transport of Cr in plants inoculated with MB + GF. A maximum of 52% reduced Cr transport was observed in the combined treatment of MB + GF along with vermicompost dose of 10 t ha⁻¹. Nevertheless, we also found that vermicompost at all the applied doses alone did not produce any impact on the translocation of Cr from roots to shoots (Fig. 6b).

Effect on uptake of NPK

Bioinoculants experiment

Data related to uptake of NPK are shown in Table 2. We found maximum uptake of N was observed in MB + GF inoculated plants. There was an increment in N uptake by 32% as compared to that of non-inoculated control. Similar trends were observed in uptake of P (38%) and K (19%) of the same treatments (Table 2).

Vermicompost experiment

The application of vermicompost doses increased the uptake of NPK content and as expected it was maximum at doses of 10 t ha⁻¹ of vermicompost. The nutrient uptake (N, P and K) uptake improved significantly (14%, 19% and 8%, respectively) as compared to the plants not treated with vermicompost. The consortia of MB + GF further improved N, P and K content in *O. basilicum* an increment of 36%, 27% and 17% uptake in N, P and K was observed, respectively, as compared to that of control (Table 3).

Microbial population estimation

In bioinoculants experiment, we found that with augmentation of Cr(VI) in soil, a constant decreased Gf colonization was observed. However, the population of GF and its colonization in bioinoculants treated plants improved significantly. The maximum increase in number of GF spores and its colonization in roots was recorded in plants inoculated with co-inoculation of MB + GF. It was found that a 95% increased spore population was observed in the pots soil treated with MB + GF (Table 4). Similarly we observed an increment of 86% colonization in the roots same aforementioned treatment.). However, we also observed that the bacterial population was not affected by either singly or

 Table 2
 NPK analysis of plant samples of Ocimum basilicum (bioinoculants experiment)

Treatments	Uptake of	ptake of NPK by plants (%)			
	0 mg kg^{-1}	25 mg kg^{-1}	50 mg kg^{-1}	100 mg kg ⁻¹	
Nitrogen (N)					
CN	1.892de#	1.791hi	1.630no	1.537p	
GF	2.138a	1.849fg	1.659 mn	1.573op	
BC	1.942bc	1.949bc	1.709jl	1.687lm	
MB	2.053ab	2.009ab	1.929bc	1.953bc	
BT	1.994ab	1.820gh	1.646mn	1.607no	
BS	1.879ef	1.756ij	1.656mn	1.617no	
BC+GF	2.063ab	2.052ab	1.934bc	1.897cd	
MB + GF	2.150a	2.085ab	2.083ab	2.023ab	
BT+GF	2.120a	2.043ab	1.683lm	1.633no	
BS+GF	1.841fg	1.871ef	1.680lm	1.643no	
Phosphorous (P)					
CN	0.756gh	0.738i	0.661mn	0.581q	
GF	0.905b	0.793ef	0.690jl	0.612p	
BC	0.868c	0.778fg	0.701j	0.628op	
MB	0.860c	0.805e	0.709j	0.649no	
BT	0.838d	0.753hi	0.676lm	0.615p	
BS	0.803e	0.757gh	0.672lm	0.615p	
BC+GF	0.926ab	0.802e	0.767gh	0.675 lm	
MB + GF	0.941a	0.831d	0.807e	0.801e	
BT + GF	0.947a	0.766gh	0.741i	0.652 mn	
BS+GF	0.912b	0.767gh	0.745hi	0.654 mn	
Potassium (K)					
CN	2.080gh	2.033hi	1.920pq	1.873q	
GF	2.353a	2.160de	1.950 mn	1.900q	
BC	2.230cd	2.210de	2.010jl	1.933op	
MB	2.223de	2.220de	2.160de	2.043hi	
BT	2.127ef	2.057gh	1.947no	1.887q	
BS	2.120fg	2.053gh	1.967lm	1.873q	
BC+GF	2.350a	2.210de	2.073gh	2.080gh	
MB + GF	2.377a	2.370a	2.327ab	2.237bc	
BT + GF	2.340a	2.123ef	2.017ij	1.890q	
BS + GF	2.333ab	2.097gh	2.017ij	1.917pq	

[#]Value showed in each column followed by different letters is significantly different at $P \le 0.01$ and the column value is the mean of four replicates

in combination treatments with GF. In the vermicompost experiment, the application of vermicompost doses further increased the GF population and it was found maximum on application of 10 t ha⁻¹ doses of vermicompost. There was also an increased spore population of 125%, was found in the soil treated with aforementioned bioinoculants as compared to that of the plants inoculated with GF alone. Like GF treatment, application of vermicompost (10 t ha⁻¹), also improved the population of MB (52%) as compared to the plants not supplemented with vermicompost (Table 5).

Treatments Uptake of NPK by plants (%) 0 t ha^{-1} 2.5 t ha^{-1} $5 \text{ t } \text{ha}^{-1}$ $10 \text{ t} \text{ ha}^{-1}$ Nitrogen (N) 1.557 h[#] CN 1.633gh 1.703fg 1.770f GF 1.563h 1.673fg 1.740f 1.873e MB 1.950de 1.990cd 2.043bc 2.113ab 2.043bc 2.070bc 2.133ab MB + GF2.187a Phosphorous (P) CN 0.6011 0.649ij 0.683 h 0.714fg GF 0.622jl 0.675hi 0.731ef 0.766d MB 0.635j 0.687gh 0.748de 0.761de MB + GF0.809c 0.856b 0.873b 0.925a Potassium (K) CN 1.907i 1.963gh 2.037f 2.057f GF 1.913hi 1.977g 2.127e 2.183cd MB 2.033f 2.073f 2.150de 2.237bc MB + GF2.190cd 2.270b 2.337a 2.370a

 Table 3
 NPK analysis of plant samples of Ocimum basilicum (vermicompost experiment)

[#]Value showed in each column followed by different letters is significantly different at $P \le 0.01$ and the column value is the mean of four replicates

Discussion

Cr(VI) stress is emerging as a major challenge to be tackled along with climate change, population expansion. There is a direct impact of Cr(VI) stress on living being and proper attention is required for devising ecofriendly management strategy. In last decade researchers have tried to manage Cr(VI) stress by isolating and enriching rhizosphere with Cr(VI) reducing rhizobacteria which could be a wise and useful option for improving plant growth yield in Cr(VI)contaminated soil (Marandi et al. 2020; Zayed and Terry 2003; Mohanty and Patra 2011; Soni et al. 2014a, b). However, there is required more efficient management strategy with an integrated approach for better management of Cr(VI) stress. In the lines of this our group looked some of the efficient options and model plants which might be employed for the management of toxicity and mitigation of Cr(VI) stress. From our previous studies we have developed ideas and demonstrated that vermicompost improves the performance of beneficial microbes (Singh et al. 2013a, b, c) and presence of chromate reducing bacteria improves AMF colonization and in turn improved plant growth (Soni et al. 2014a). Vermicompost apart from acting as a source of nutrients also have ability to chelate (Pande et al. 2007) and reduce Cr(VI) (Koz'uh et al. 2000) toxicity. The compound such as humic substances and polysaccharides present in vermicompost act as a natural chelating agent which may link heavy metals (Bianchi and Ceccanti 2010) and helps in

 Table 4 Mean population of microbes in the root zone soil of Ocimum basilicum at the time of harvesting

Treatment	Root zone microbial Population				
	SUCR inocula	Arbuscular my	corrhizal fungi		
	$(CFU \times 10^5 \text{ g}^{-1} \text{ soil})$	No. of spore $(50 \text{ g}^{-1} \text{ soil})$	Percent root colonization		
0 mg kg ⁻¹					
CN	_	_	_		
BC	1.52ab#	_	_		
MB	1.66ab	_	_		
BT	1.41cd	_	_		
BS	1.31ef	_	_		
BC+GF	1.50bc	236ab	66ab		
MB+GF	1.69a	243a	65ab		
BT+GF	1.43cd	230ab	69a		
BS+GF	1.28ef	229ab	66ab		
GF	_	241ab	68ab		
25 mg kg ⁻¹					
CN	_	_	_		
BC	1.30ef	_	_		
MB	1.59ab	_	_		
BT	1.27ef	_	_		
BS	1.21fg	_	_		
BC+GF	1.32de	214bc	60cd		
MB+GF	1.57ab	230ab	62bc		
BT+GF	1.30ef	186ef	56ef		
BI+GF BS+GF	1.19fg	178fg	52fg		
GF	1.191g	•	45hi		
50 mg kg ⁻¹		162gh	45111		
CN					
BC	— 1.26ef	—	—		
MB		—	_		
	1.51ab	—	—		
BT	1.17fg	—	—		
BS	1.10gh	-	-		
BC+GF	1.22fg	197de	50fg		
MB+GF	1.49bc	222ab	56de		
BT+GF	1.26ef	154hi	47gh		
BS+GF	1.13fg	138ij	43i		
GF	_	122lm	35j		
100 mg kg ⁻¹					
CN	_	—	—		
BC	0.81j	—	—		
MB	1.08hi	_	-		
BT	0.67jl	_	_		
BS	0.581	_	_		
BC + GF	0.81j	172fg	44i		
MB + GF	1.04i	205cd	52fg		
BT + GF	0.70j1	135jl	32j1		
BS+GF	0.551	124lm	30j1		
GF	_	105m	281		

[#]Value showed in each column followed by different letters is significantly different at $P \le 0.01$ and the column value is the mean of three replicates

Treatment	Root Zone microbial Population				
		Arbuscular mycorrhizal fungi			
	soil)	No. of spore $(50 \text{ g}^{-1} \text{ soil})$	Percent root colonization		
0 ton/ha					
CN	_	_	_		
MB	1.09d [#]	_	_		
GF	-	110e	29e		
MB + GF	1.10d	214b	49c		
2.5 ton/ha					
CN	-	-	_		
MB	1.27c	-	_		
GF	-	150d	39d		
MB + GF	1.24cd	227b	55b		
5 ton/ha					
CN	-	-	-		
MB	1.46b	-	-		
GF	-	133cd	45c		
MB + GF	1.43b	225b	57ab		
10 ton/ha					
CN	-	-	-		
MB	1.66a	_	_		
GF	-	167c	50c		
MB + GF	1.69a	248a	61a		

 Table 5
 Mean population of microbes in the root zone soil of Ocimum basilicum at the time of harvesting

[#]Value showed in each column followed by different letters is significantly different at $P \le 0.01$ and the column value is the mean of three replicates

mitigation. However, we were looking for the model plant along with integrated management approach, which will not only mitigate the Cr(VI) stress, but products obtained can also be fit to be used for. Therefore, we devised a study, selecting *O. basilicum* as a model plant and hypothesized that vermicompost as a substrate will improve the population of chromate reducing rhizobacteria and in turn mycorrhiza colonization leading to enhanced growth of plants in chromate polluted soils besides this *O. basilicum* being an aromatic plant may act as a good model as its secondary metabolite (essential oil) are not impacted by Cr(VI) stress, except may be in quantity.

The screening results for the better performing strains revealed that only MB and its combination with GF were effectively mitigating the Cr stress. While treatments with other microbes, i.e., BC, BF alone and in combination were lower in responses (Tables S1–S9, supplementary files) as compared to that of MB. Since all the strains cannot be assessed for biopotentials due to technical handling we narrowed our studies to the most efficiently performing strains. Such selection criteria for microbial selection based on performance is not only followed in abiotic stress management but also in biotic stress management (Soni et al. 2014a; Tiwari et al. 2021). The results from our studies clearly demarcated that the plant growth (root length and shoot height) and yield (dry biomass) were found to be reduced when plants were cultivated under Cr(VI) stress. The impact of severity further increases with increase in the concentration of Cr(VI) as our results revealed the same with gradient concentration used in. The major impact of toxicity occurs on the root system of the plants. Our studies also demonstrated that root growth and development is hampered. The reduction in plants root growth could be due to disruption in cell division process which causes time lengthening of cell cycle. Apart from aforementioned, the direct Cr contact with root may damage the plasma membrane of root cells and consequently leakage of cell content might be increasing the severity, leading to collapse of root system (Castro et al. 2007). We also found that there was drastic reduction of plant growth yield in untreated plants, which increased with concentration gradient. The reduction in plant height and dry biomass could be due to reduction in nutrient uptake and further might be a possibility that transport of water and nutrients system have collapsed due to poor developed root system which is essential for supporting plant growth and development (Shankar et al. 2005). Moreover, Cr transport to aerial parts may also have a direct impact on cellular metabolism of shoots causing reduction of plant growth (Shanker et al. 2005). Besides this, Cr also interacts with endogenous phytohormones and inactivates them (Moya et al. 1995). Our studies also found that the decline in yield (dry biomass) was also increased with exposure to increased gradient concentrations of Cr(VI). The decrease in dry biomass production might also be due to the oxidative damage (due to ROS generation) of the photosynthetic and mitochondrial apparatus/processes which plays vital role in growth development and yield (Dixit et al. 2002). However, we have found that with the application of bioinoculants and vermicompost along with mycorrhiza have potentials to manage toxicity of Cr(VI) and even have contributed to growth yield and development. Our results demonstrated that with application of chromium tolerant microbes such as MB, in O. basilicum potentially helped in mitigating toxic impacts of Cr(VI) and supported growth. This could be due to conversion of toxic Cr(VI) to relatively nontoxic Cr(III) in rhizospheric environment thus providing relatively favorable conditions for growth of plant roots GF and thus facilitating nutritional uptake (Soni et al. 2014a). We also found that combination, co inoculation are better options for the stress management, not only related to abiotic stress but even biotic stress can be managed better with co-inoculation of one or more microbes in synergism (Tiwari et al. 2017, 2021). Here we also found that combination of microbes MB + GF performed better in management of Cr(VI) stress than comparison to singly inoculated treatments. Our result suggest that the application of MB can lower the chromium toxicity to the plant by reducing the bioavailability of toxic Cr(VI). The reduced Cr(VI) toxicity levels in soil may provide the favourable environment for the growth, proliferation, and colonization of AMF resulting in improved growth and yield of crop plants. Moreover, improved colonization of AMF can lower down the heavy metal toxicity to plants by immobilizing the metals and further restrict the translocation of Cr(VI) to aerial parts.

Further on, application of vermicompost as we said previously has self-potentials of heavy metals (Jadia and Fulekar 2008) including Cr(VI) stress management (Koka et al. 2019). We found that microbial co-inoculation performed much better and improved growth, yield and nutrient content then comparison to microbes alone or vermicompost treatments in alone. Our results further supported the model of integrated approach of management, as demonstrated that combination of vermicompost + MB + GF have showed much improvement in plant growth and yield then comparison to any other treatments. This might be due to vermicompost acted as a substrate for proliferation of bacterial population, thus enriched the rhizosphere with chromate reducing bacteria, second vermicompost itself helped in GF multiplication and colonization, which is well known for its potentials in abiotic stress management (Khan et al. 2001). The effective microbial treatments (MB+GF) also enhanced nutrient uptake (NPK) in treated plants. This could be due to the reason that they might have reduced toxicity of the Cr (VI) which directly impacted in better root system development and nutrient absorption. Apart from this vermicompost itself has been found as a supplement for plant nutrients (Kumar and Sharma 2018), not only this they maintain moisture content of the soil, They have adsorbing capability for heavy metals thus may reduce their availability to the plants reducing severity and toxicity (Matos and Arruda 2003; Jordão et al. 2009, 2010, 2011; Koka et al. 2019). However, the heavy metal adsorption capacity of vermicompost depends on its particle size, porosity, as well as the molecular structure of the adsorbed compound (Lamim et al. 1998). In our study they performed well which supports the previous results that vermicompost itself have Cr(VI) mitigating potentials.

However, the most commonly reported mechanisms for metal ion sorption are electrostatic interaction, ion exchange, complexation and precipitation (Barrera et al. 2006) which might have worked in our experiment and helped in mitigation of Cr(VI) stress. There is also another strategy of management by vermicompost which employs humic and fulvic acid present in them (Chaab et al. 2016), have demonstrated potentials which can also reduce the Cr(VI) and may indirectly help in improving root growth (Koz'uh et al. 2000). Moreover, there could be also a possibility of alleviation of plant toxicity due to Cr chelation by vermicompost (Pande et al. 2007). Therefore, these all factors might have contributed well in Cr(VI) stress management on O. basilicum as demonstrated by our results. Our results also showed there is decline in the bioavailability of Cr(VI) which might be due to the higher amount of Cr reducing metabolites produced by MB (Soni et al. 2013) although the possibility of adsorption of Cr(VI) by these strains cannot be neglected (Kanga et al. 2007). Not only this the integrated management approach involving vermicompost might also be a major player and contributed well in reducing the bioavailable Cr(VI) in soil. As our results showed lower bioavailable Cr(VI) in combined treatments. Therefore, possibility of vermicompost cannot be neglected in reducing the bio-availability of Cr in soils. Furthermore, the application of vermicompost also reduced the uptake of Cr. This could be due to the direct conversion of available Cr(VI) into non available forms either by reduction or chelation of Cr by vermicompost. There are reported literature which also emphasized that microflora and organic compounds such as vermicompost plays critical role in, the conversion of Cr(VI) into Cr(III) which also gets further enhancement with texture, and conditions, i.e., pH, moisture, temperature, and oxidation-reduction conditions, in the particular soil (Koz'uh et al. 2000; Polti et al. 2009). Therefore, we predict that these factors might have influenced Cr(VI) reduction and showed direct impact on Cr uptake by plant cells in soil.

We have found that with the treatment of bioinoculants there is reduction of translocation factor. They performed better in combination treatments. The decrease in translocation of Cr from root to shoot could be due to immobilization of Cr in vacuole of root cells (Shanker et al. 2005). Furthermore, we also found that the application of vermicompost also reduced the translocation of Cr from root to shoot. The decrease in translocation may be because of improved of root growth due to reduced chromate toxicity or indirectly by increase in population of rhizobacteria and mycorrhiza. All these factors might have contributed in lowering the uptake and toxicity. There is also a well reported mechanism of mycorrhizal association benefits plants by coping with abiotic stresses. The presence of mycorrhiza in roots will immobilize metals in several ways such as binding of heavy metals to chitin present in fungal cell walls. In addition, fungal vesicles may be involved in storing toxic metals and thereby avoiding their translocation to upper parts of the plants (Gother and Paszkowski 2006).Since vermicompost enhanced the mycorrhization and colonization, they might have played role in lowering the toxicity by sorting or immobilizing metals and reducing translocation thus lowering the toxicity to the plants.

Conclusions

This study clearly demonstrated the successful role of vermicompost in reducing Cr toxicity in plants. The experimental evidence showed that vermicompost consequently reduced Cr(VI) toxicity with gradient doses of application. It further decreased the bioavailability and uptake of Cr to the upper parts of the plants. The vermicompost not only directly but even indirectly supported management of Cr(VI) stress by supporting the proliferation of chromate reducing rhizobacteria such as MB, that further decreased the bioavailable and toxic form of Cr(VI) and consequently contributed to the higher herb yield. Furthermore, the integrated approach also improved mycorrhization and contributed in synergistic management of Cr(VI) stress. With the application of the integrated management approach, a new model for management of Cr(VI) stress has been paved. This model will not only benefit the Cr(VI) stress management in O. basilicum but will also guide framing such approaches in other crops and plants too.

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Author contributions SKS and RS designed the experiment and conducted the experiment; ST and RS helped in data analysis and editing of the original draft. SKS and ST prepared the final draft. All authors have read and agreed with the present version of the manuscript.

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Declarations

Conflicts of interest The authors declare that there is no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication All authors have seen the latest version of the manuscript and agree to its publication.

Ethics approval and consent to participate Not applicable.

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