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ULTRAVIOLET TREATED ASPERGILLUS TERREUS SH 13 DETOXIFIES HIGHER LEVELS OF CHROMATE AND MITIGATES CHROMATE STRESS IN *BRASSICA NAPUS* BY REDUCING EXPOSURE OF HOST TISSUES

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Abstract

Contamination of agricultural lands by the heavy metal is a serious biological and environmental issue. Such a hazard can be minimized and confronted by enhancing the growth promoting and chromium alleviating competences of fungal endophytes. Current attempt was made to find the effect of ultra violet (UV) irradiation on plant growth promoting and Cr alleviating capabilities of fungal endophytes. For the said purpose spore suspension of various fungal endophytes were exposed to UV irradiation for various time points i.e., 15, 30 and 45 min. After exposure to UV, the strains were screened for their bio-remediating abilities. Among the UV irradiated fungal endophytes, the growth promoting and Cr alleviating capabilities of Aspergillus terreus strain SH 13 was significantly (P=0.05) increased in comparison to its wild type. The elevated level of Cr in the soil resulted in growth inhibition of the seedlings. However, the UV irradiated SH 13 isolate has restored the growth parameters of the seedling in the said Cr stress. The increase in host plant lengths was almost 2 folds greater than those supplemented with non-UV irradiated SH 13 strain. The UV treated isolate effectively cut down the absorption of Cr from soil to root, allowing the seedlings to resume their normal growth and functions. Moreover, the strain exhibited extensive ability to strengthen the host antioxidant system by lowering ROS accumulation, improving ROS scavenging, and promoting plant's antioxidant contents (phenol and flavonoids). The above findings suggest that UV exposure can enhance growth promoting and bio-remediating (Cr reclamation) abilities of *A. terreus* SH 13.

Key words: UV irradiation, *Aspergillus terreus*, Bioremediation, ROS accumulation.

Introduction

In the ecosystem, it is a routine exercise that variety of mutants is produce through accidental and random mutagenesis. Now-a-day, the phenomenon of mutagenesis is usually implicated for the improvement of microbial strains, but induced mutagenesis is a trial-and-error process, which is very tedious and laborious. Moreover, improved physiological processes and performance by mutant strains is always a black box i.e., the undergoing mechanisms are not easily identified (Tong & Feng, 2022). Production of lesions or modification in DNA sequences indicate mutation that may happen after exposure to UV, X-rays radiations or ethyl methane sulphonate, etc. The production of un-repairable lesions might suggest successful mutation (Ainsworth & Gillespie, 2007). From the past experiences, we know that mutations are not always beneficial; therefore, extensive screening is required to develop a desirable phenotype. In order to induce random mutation in organisms, different modes and methods have been introduced, such as exposure to UV radiations, nitrous oxide, ethyl methane sulphonate, etc. (Takahashi *et al.*, 2001). UV radiations and heavy metal stress are well-known to increase the accretion of secondary metabolites in plants (Vanhaelewyn *et al.*, 2020). Carbon flux can be redirected by UV light thus resulting in a positive change in variety of metabolites like phenols, carotenoids and glucosinolates (Heinze *et al.*, 2018). Having similar secondary metabolic features with their host plants, it is possible that endophytic fungi may also accrue secondary metabolites by exposing them to UV radiations and thus their growth promoting abilities may be enhanced under heavy metal stress.

Metallic elements with higher density (4.5 gm/cm^3) and exhibiting toxicity even at smaller concentrations are categorized as heavy metals (HMs). Under recommended levels some of the heavy metals are not hazardous and have positive role in the routine activities of plants and animals (Wintz *et al.*, 2002). HMs are required by the plant species at very low concentrations for their proper growth, however, exposure to higher concentrations result in poor yield (Qadir *et al.*, 2022). Accumulation of high HMs in crops can affect humans and other animals either directly or indirectly as entering in to they enter their food chain (Wieczorek-Dąbrowska *et al.*, 2013). Also, it might affect the cellular machinery like organelles, enzymes and metabolites that are known for detoxification and cellular recovery from injury (Wang *et al.*, 2001). HMs can damage various proteins and DNA of the cell, thus resulting in modulation of cell cycle, oncogenesis and apoptosis (Beyersmann & Hartwig, 2008). Among the HMs, hexavalent chromium (Cr^{+6}) is considered as the most notorious industrial contaminants. Cr^{+6} are responsible for mutagenesis, carcinogenesis and phytotoxicity at above its threshold level (Ackerley *et al.*, 2006). Being highly soluble, chromium can effortlessly enter the water

resources and finally ends up in food chain (Sharma *et al.,* 2020). Crop species growing in the contaminated soils absorbs high levels of Cr^{+6} that reduces roots and shoots biomasses, crop yield, inhibits seed germination and quality of flowers (Majhi *et al.,* 2020). Enhanced production of ROS is among the hallmarks of Cr^{+6} toxicity that causes oxidative modifications of proteins, lipids and nucleic acids (Lushchak *et al.,* 2011). The extreme toxicity and oncogenic properties of Cr^{+6} are due to their high penetrability through the biofilms and their interface with the proteins and genetic material (Bayramoglu *et al.,* 2005).

As HMs badly affect the crops, animals and humans, its remediation through cheap and rigorous methods is very necessary (Tchounwou *et al.,* 2018). Bioremediation is one of the contemporary practices used for the elimination of HM's. Endophytic fungi are considered as the best candidate for the bioremediation of heavy metals as they hold variety of mechanisms to detoxify the metals (Gadd *et al.,* 2014: Musa *et al*., 2023). The aim of current study was to inspect the effect of UV-C on the survival, growth promoting capabilities, metabolites and phytohormones production of *A. terreus*; ability of the UV-C irradiated strains to mitigate Cr^{+6} alleviation in *Brassica napus* L.; efficacy of the UV-C irradiated *A. terreus* to strengthen host's antioxidant system and ROS scavenging ability; comparative study of the wild SH 13 strains with UV-C irradiated SH 13 strain.

Material and Methods

Plant of *Euphorbia helioscopia* growing in the industrial area of Mardan were uprooted and brought to plant microbe interaction laboratory, department of botany, Abdul Wali Khan University Mardan. Parts of the plant were cleaned with running tap water. Surface sterilization was done in 70% ethanol for 1 minute, and then dipping in 2% hypochlorite (NaOCl) solution for 3 min. Finally the plant parts were carefully washed off with distilled water (Stone *et al.,* 2004). Segments (1-3 cm) of the cleaned and surface sterilized plant were placed on hagum minimal media in the fume hood under sterilized conditions. Plates were then incubated for 7 days at 30°C. In order to attain pure colonies, the isolated fungal strains on hagum media were sub-cultured on the PDA (potato dextrose agar).

Cr resisting and plant growth promoting assay: A group of isolated endophytic fungi were tested for their growth promoting and Cr alleviating potency in *Brassica napus*. For the said purpose, uniformly germinated *B. napus* seedlings were transferred to sterilized soil supplemented with different concentration of Cr (i.e., 25 ppm and 50 ppm) and fungal biomass. After growing for a period of 14 days, seedlings were harvested and their shoot, root lengths, fresh weight, chlorophyll and carotenoid contents were recorded.

Molecular identification of fungal isolate SH 13: Freeze mycelia was used to isolate DNA for molecular identification (Li *et al.,* 2015). About, 200 mg of fungal biomass was ground in 500 µL buffer (sodium-dodecyl-sulfate, 0.1 M NaCl, and 0.5 M Tris-HCl) at pH 8 and vortexed for 10-15 s. The aforementioned mixture was heated at 65˚C for 25 min and then centrifuged at 7500 rcf for 5 min. Phenol-chloroform-isoamyl alcohol (25:24:1) was added to the supernatant at the ratio of 1:1 and centrifuged at 7500 rcf for 5 min. The aqueous layer was mixed with chloroform-isoamyl alcohol (24:1) and centrifuged for 5 min at 7500 rcf. The collected supernatant was mixed with ethanol and incubated for 50 min at 4°C followed by centrifugation at 7500 for 15 min to precipitate DNA. The DNA was amplified with ITS1 and ITS4 regions using PCR. The whole PCR reaction blend consists of 15 µL PCR master mix, 1.5 µL of DNA sample, 1.5 µL of each primer and 10.5 µL of double distilled water. PCR amplification of target DNA regions was done by denaturation at 95°C for 2 min, annealing at 55°C for 1 min, and extension for 5 min at 72°C. To achieve an amplified DNA fragment, 35 cycles were performed. The gel purified PCR fragment was subjected to sequencing reaction. The resulted sequence reads were aligned and homology of the consensus sequence was assessed through nucleic acid blast (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences possessing highest homology with the isolate was recovered to know the precise nomenclature of the isolate. MEGA-7 was used for phylogenetic analysis of the sample (Chun *et al.,* 2007; Tamura *et al.,* 2011).

UV mutagenesis and screening of Cr^{+6} resistant *A. terreus*: For the purpose of UV mutagenesis, spore suspension of the endophytic fungi was prepared by scraping spores from the colony surface and suspending them in sterilized distilled water. Afterwards the spore suspension was diluted to 1×10^7 spores per ml by counting the spores in hemocytometer, and was then used as an inoculum, which was subjected to UV-C light. The prepared spore suspension of the selected endophytic fungus *A. terreus* (SH 13) was discharged into the autoclaved Petri plates. The plates were then placed under UV-C source (12 cm) for different exposure time, i.e., 15, 30 and 45 min. The whole irradiation process was carried in complete dark to avoid photo-reactivation. After each time point of UV treatment, 1.5 ml of irradiated spore suspension was drawn and serially diluted up to 10 folds with sterilized distilled water. A tiny drop of diluted spore suspension was then discharged in Petri plates containing potato dextrose agar medium and incubated at 28°C for 3 days till sporulation of fungal culture. Mycelia were randomly selected and placed on potato dextrose agar for further analysis. Czapek broth media supplemented with different concentrations of Cr^{+6} (300 ppm, 600 ppm and 900 ppm) was taken as the screening media for the selected UV-C irradiated strains of endophytic fungi. Growth of the endophytic fungi (fresh and dry weight of fungal mycelia) was recorded after incubation of the mutated fungal strains in shaking incubator for 7 days. The UV irradiated strains were also grown on potato dextrose agar (PDA) media and incubated for 7 days in the incubator at 30°C to study their colony morphology.

Morphology of the fungal strains: Slide culturing technique was used to examine the morphology of the endophytic fungi and its mutant (Anthony & Walkes, 1962). A block of potato dextrose agar was mounted on the slide under sterilized condition and was inoculated with fungal spores from all four sides. The block of potato dextrose agar was covered with a cover slip to avoid surface growth. Slides were placed in sterile petri plates, followed by 3 days incubation at 30°C. The growing endophytic fungi were then stained with lacto phenol cotton blue dye and were visualized at 40X using a light microscope.

Determination of fungal (wild type and their mutants) metabolites: Calorimetric bioassay was performed in order to quantify the amount of IAA, salicylic acid flavonoids, and phenolic contents in fungal culture filtrate (FCF) of wild type and UV-C irradiated strains. FCF was obtained by filtering fungi grown Czapek broth through Whatman No.1 filter paper.

Indole acetic acid (IAA): Salkowski reagent was used to find the amount of IAA in FCF via spectrophotometer (PerkinElmer Lamda 25 double beam spectrophotometer) at 540 nm (Hussain *et al.,* 2011).

Salicylic acid (SA): Method of (Warrier *et al.,* 2013) was used to determine the amount of SA in FCF. Freshly prepared 1% ferric chloride (5.8 ml) was added to 0.2 ml of FCF and the absorbance was recorded at 540 nm.

Flavonoids: Estimations of flavonoids was done by means of $AlCl_3$ method (El Far *et al.,* 2009). To 0.25 ml FCF, 50 µL $AlCl_3$ (10%), 50 µL potassium acetate (10%) and 2.4 ml methanol (80%) was added. After combining the contents of the mixture, it was robustly shaken and incubated for 25 min at 25˚C. Absorbance was recorded at 415 nm against methanol as a blank.

Phenolic content: Phenolic contents in FCF was measured using a revised method of (Ainsworth & Gillespie, 2007). To 100 µL of FCF, 1.4 ml of double distilled water and 0.25 ml of Folin-ciocalteu reagent was added at the same ratio (1:1). After incubation for 4 min at 26˚C, 1 ml of 20% sodium carbonate was added. The final mixture was heated for 1 minute and upon cooling, absorbance was recorded at 650 nm.

Screening of wild type and UV-C irradiated *A. terreus* for growth promotion and Cr^{+6} alleviation in *Brassica napus*: Seeds of *B. napus* were sterilized with 0.1% $HgCl_2$ for 6 min, and were then rinsed three times with autoclaved distilled water. The disinfected seeds were uniformly positioned in pots containing 300 g of sterilized and autoclaved soil. Randomized complete block design (RCBD) was carried for pot experiment and each treatment was replicated 3 times with 10 sterilized seeds per treatment. Upon germination, the non-uniform *B. napus* seedlings were removed and the uniform ones were left in the pots. The experimental layout was as follows:

T1 = *B. napus* seedlings (control)
T2 = *B. napus* seedlings + 25 ppm Cr^{+6}
T3 = *B. napus* seedlings + 50 ppm Cr^{+6}
T4 = *B. napus* seedlings + wild *A. terreus* SH 13
T5 = *B. napus* seedlings + wild *A. terreus* SH 13 + 25 ppm Cr^{+6}
T6 = *B. napus* seedlings + wild *A. terreus* SH 13 + 50 ppm Cr^{+6}
T7 = *B. napus* seedlings + UV-C irradiated *A. terreus* SH 13
T8 = *B. napus* seedlings+UV-C irradiated *A. terreus* SH 13 + 25 ppm Cr^{+6}
T9 = *B. napus* seedlings + UV-C *A. terreus* SH 13 + 50 ppm Cr^{+6}

All the pots were placed in field during winter under natural condition and were daily irrigated with 50 ml water per pot. Growth parameters of the plants were recorded after 28 days of germination.

Root colonization: Roots of *B. napus* seedlings were stained with lacto phenol cotton blue in order to study the fungal mycelia and hyphae under light microscope (Larone & Larone, 1987). Briefly, a few drops of lacto phenol cotton blue were added to the root section positioned on the glass slide. After 3-4 min, excess stain was removed by washing the root section with 70% ethanol, followed by washing with distill water (twice). In order to visualize fungal hyphae, fine sections of the target roots were placed on the glass slides and were analyzed under light microscope using 40X objective lens.

Total pigments analysis: Leaves of the plants under Cr^{+6} stress were subjected to pigment analysis using spectrophotometer (Sumanta *et al.,* 2014). Fresh leaves (0.5gm) were crushed in 5 ml of 80% acetone. Absorbance of the ground samples were recorded at 645 nm and 663 nm (Perkin Elmer Lambda 25 Double Beam Spectrophotometer). Carotenoids contents in the samples were also recorded at 480 nm (Kirk *et al.,* 1965).

Metabolites profile of the chromate stressed seedlings

Total flavonoids and indole acetic acid: For the extraction of total flavonoids, 1 gm of seedlings was crushed in 10 ml of 80% ethanol and was left overnight in the shaking incubator at 25°C. Supernatants from the extract was then restored by centrifugation at 7500 rcf for about 15 min and was stored in 25 ml falcon tubes at 4°C. For the estimation of total flavonoids, $AlCl_3$ method was used as described earlier (El Far *et al.,* 2009). Total indole acetic acid in the leaves extract were measured using salkowski reagent method as discussed earlier.

Salicylic acid: Extraction of salicylic acid from the seedling was done by grounding 0.5 gm of seedling in 5 ml of 80% ethanol. The ground sample was left overnight in a shaking incubator at 28°C. Supernatants from the extract was restored by centrifugation at 7500 rcf for about 15 min. The supernatant was collected and stored at 4°C. Salicylic acid in extract was measured using aforementioned method (Warrier *et al.,* 2013).

Total phenol content: Phenolic contents were estimated by homogenizing 1 g of seedlings in 10 ml ethanol.

Homogenized sample was incubated at 40°C for about 3 h. The mixture was then centrifuged at 7500 rcf for 15 min at 25˚C. After careful isolation of supernatant, it was re-dissolved in 10 ml distilled water and stored at 4°C prior to analysis. Estimation of phenolic contents in the extract was done using the methodology of (Ainsworth & Gillespie, 2007) as discussed earlier.

Antioxidant enzyme activities in *Brassica napus* under Cr stress

Peroxidase activity: Peroxidase activity was carried out using guaiacol as a substrate for dehydrogenation. For the purpose of enzyme extraction, 0.2 g of the plant sample was grounded in 6 ml of 0.1 M phosphate buffer (PH 7.0) using pistil and mortar, following centrifugation for about 15 min at 7500 rcf. The total reaction blend included 0.2 ml of enzyme extract, 0.1 M phosphate buffer (6 ml) at pH 7, 0.06 ml of 12.3 mM hydrogen peroxide, and 0.1 ml of 20 mM guaiacol (Malik *et al*., 1980). After vigorous shaking of reaction mixture in cuvette, alteration in the optical density by 0.1 (t) was documented at 436 nm. The POD activity was calculated by the following formula:

Peroxidase enzymatic activity:

$$= \left(\frac{500}{\Delta t}\right) \times \left(\frac{1}{1000}\right) \times \left(\frac{TV}{VU}\right) \times \left(\frac{1}{f.wt}\right)$$

In the given formula;

Δt = Time change (per minute),
TV = Total volume of extract
VU = Volume used (Sousa *et al.,* 2012)
F wt = Fresh weight of leaf tissue in (g)

Catalase activity: Catalase activity the leaves of *B. napus* was determined by the method of (Huseynova, 2012). A total of 2.6 ml of potassium phosphate buffer (50 mM) and 40 µL enzyme extract was mixed uniformly (as described above). The mixture was supplemented with 0.4 ml of H_2O_2 (15 mM) and the absorbance was recorded at 240 nm against the blank.

Ascorbate peroxidase activity: Ascorbate peroxidase activity (APX) was evaluated according to the method of (Asada, 1992). To 0.3 ml enzyme extract, 900 µL of potassium phosphate buffer (50 mM, PH 7.0), 150 µL of ascorbic acid (0.5 mM) and 150 µL of H_2O_2 (0.1 mM) was added. The absorbance was noted at 290 nm after every 30 s for 5 min against the blank.

1,1-diphenylhydrazyl assay: 1,1-diphenylhydrazyl **(**DPPH) radical scavenging activity was carried out using the technique of (Meng *et al.,* 2016).Briefly, 0.2 gm of plant leaves was homogenized in 2 ml of methanol and centrifuged at 7500 rcf for 10 min. DPPH solution was made by dissolving powdered DPPH in methanol (0.004%). Plant sample (1 ml) was mixed in 2 ml DPPH solution and was incubated in dark for about 30 min. Absorbance was finally recorded spectrophotometrically at 517 nm against the blank.

Analysis of heavy metal Cr using atomic absorption spectroscopy

BCR sequential method: The estimation of two different species of heavy metal Cr in plants tissue and fungal culture filtrates was done using the technique of community bureau of references (BCR) (Kazi *et al.,* 2005). The extraction was done in three key stages.

1) Acid soluble and exchangeable: Air dried sample (0.5 gm) was mixed with 20 ml of 0.11 M acetic acid (Sigma Aldrich, Burlington, MA, USA). The mixture was placed in a shaking incubator for 24 h at 25-30°C. After 24 h, the residue and supernatant were separated using centrifugation at 675 rcf for 3 min at room temperature.

2) Reducible fraction: The residue from step 1 with the 20 ml of 0.5 M hydroxyl-amine hydrochloride (pH1.5). The mixture was shaken for about 16 h at 30°C. The residue and supernatant were separated using the procedure mentioned in step 1.

3) Oxidizable fraction: The leftover residues from step 2 were mixed with 5 ml of 30% hydrogen peroxide (H_2O_2) of pH2. The mixture was continuously shaken for an hour and fully dehydrated using evaporation. In order to achieve precise results, washing and dryness of residues was done twice. After thorough washing, the dried residue was re-suspended in 25 ml of 1 M ammonium acetate (CH_3COONH_4) and kept for incubation for a period of 16 h at 28°C.

At each step of the extraction, the pellets were thoroughly washed, shaken for 15 min, and then centrifuged for 20 min at 504 rcf. Deliberate washing was done to achieve accurate measurements and avoid any contaminations from residues.

Atomic absorption spectroscopy (AAS)

Measurements were done using Flame atomic absorption spectroscopy (PerkinElmer AAnalyst 700 USA) with air/ acetylene flame under standard operational procedure as recommended.

Bio-concentration factor (BCF): Using the given equation, heavy metal (Cr) accreted in the samples were measured.

$$\text{BCF} = \frac{\text{Metals in biomass}}{\text{Metals added to media}}$$

ROS visualization using DAB: In order to visualize the accumulation of total ROS in the leaves, 3,3′-di-aminobenzidine (DAB) was used following the methodology of (Jambunathan, 2010). Briefly, leaves were immersed in DAB solution with continuous shaking at 100 rcf for about 3 hours to infiltrate the solution. Surplus dye was washed with the bleaching solution made by mixing ethanol, acetic acid and glycerol at the ratio of 3:1:1. Leaves were then heated in ethanol in order to remove the chlorophyll content using water bath. The stained leaves were then visualized using light microscope.

Statistical analysis: To achieve viable and precise results, all the experiments and assays were repeated thrice keeping constant and uniform environmental conditions and laboratory procedures. Analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was performed at $p \leq 0.05$ significance level using SPSS software (IBM SPSS Statistics21). Graphs were plotted using Graph Pad Prism (Version 5.03).

Results

Isolation of chromium resistant Endophytic fungi: A set of different endophytic fungi were isolated from all parts of *E. helioscopia.* Amongst the isolated endophytic fungi, SH 13 was selected for its ability to promote growth of *B. napus* and mitigate chromate stress. In the presence of 25 and 50 ppm chromate, SH 13 associated seedlings achieved better shoot and root growth than the control seedlings (Fig. 1).

Identification of the target strains: Morphological features of the selected endophyte were studied. Fungal strain SH 13 appeared to be a fast-growing filamentous fungi having light brown color. The strain was able to produce large number of conidia on the conidiophores. The conidia were spherical to oval in shape (Fig. 2).

Identification on the basis of ITS sequencing and phylogenetic analysis: Fungal strain SH 13 was identified using ITS rDNA sequencing and phylogenetic analysis. To recognize the genus or specie, the identified sequence was matched with available data in the GenBank sequence database. Homology search on GenBank classified the fungal strain SH 13 up to genus level and showed greater resemblance with *Aspergillus terreus* (Fig. 2).

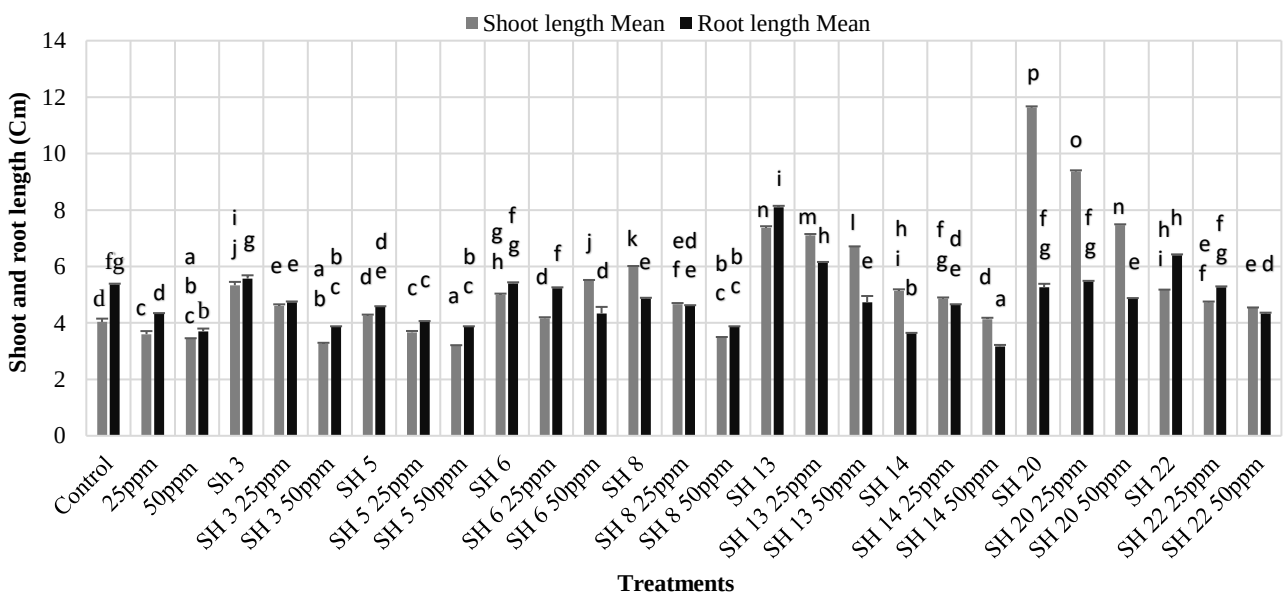

Fig. 1. Effect of different concentration of chromium and endophytic fungi on the growth attributes of *Brassica napus.* The seedlings were allowed to grow for 14 days in plastic pots containing autoclaved soil spiked with 25 and 50 ppm of chromate. Data are the mean of 3replicates with standard error.

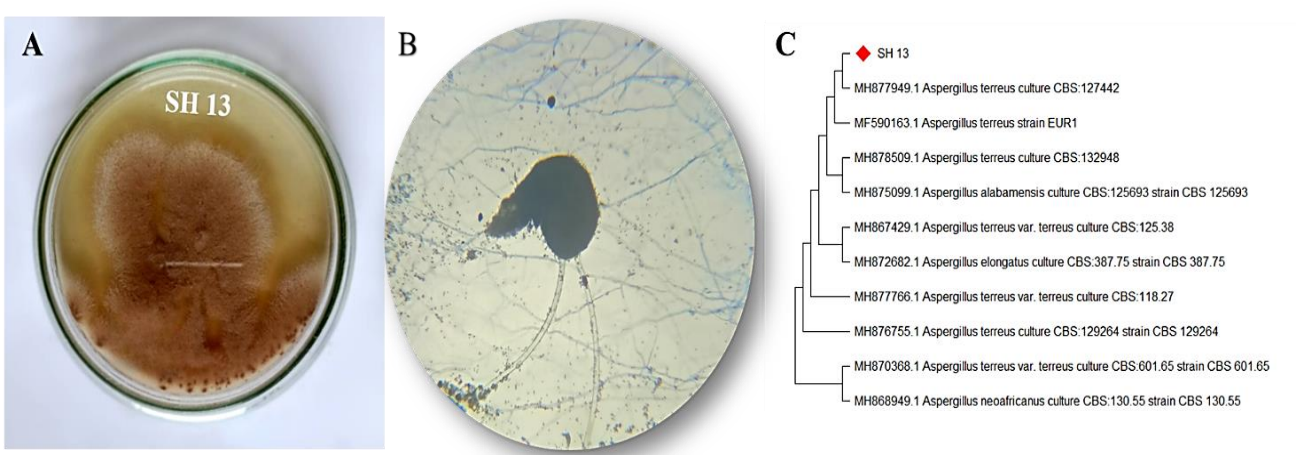

Fig. 2. Characterization of chromate resistant endophytic fungus *Aspergillus terreus* SH13; colony morphology on PDA (A) light microscopy of the lactophenol cotton blue stained hyphae and conidiophore (B) and Phylogenetic association depicted by a neighbor joining tree reflecting ITS rDNA sequence homology of the isolate with closely matching sequences retrieved from NCBI GenBank (C).

Table 1. Comparison of growth of wild type (SH 13) and mutant strains in Czapek broth containing different concentrations of Cr^{+6}. Among all the variants SH 13 (30b) showed promising growth even in higher concentrations of Cr^{+6}.

Mutant strain	*Aspergillus terreus* /100ml Czapek Dox Broth			
	Mutagen agent and exposure time (min)	Fungal biomass (g) at 300 ppm (Cr^{+6})	Fungal biomass (g) at 600 ppm (Cr^{+6})	Fungal biomass (g) at 900 ppm (Cr^{+6})
Wild type SH 13	-	9.136 ± 0.03844^{p}	8.833 ± 0.01453^{o}	8.136 ± 0.04333^{m}
SH 13 (15 a)	UV 15	8.166 ± 0.02603^{i}	6.233 ± 0.02848^{e}	4.386 ± 0.01764^{cd}
SH 13 (15 b)	UV 15	8.303 ± 0.00333^{kl}	5.573 ± 0.01202^{c}	5.136 ± 0.04667^{f}
SH 13 (15 c)	UV 15	7.273 ± 0.01764^{de}	5.843 ± 0.02906^{d}	5.160 ± 0.02082^{f}
SH 13 (15 d)	UV 15	8.913 ± 0.00882^{o}	5.183 ± 0.01333^{a}	4.260 ± 0.02646^{c}
SH 13 (15 e)	UV 15	8.830 ± 0.01528^{n}	5.613 ± 0.03180^{c}	4.486 ± 0.02028^{cde}
SH 13 (15 f)	UV 15	6.426 ± 0.01202^{a}	6.753 ± 0.03180^{g}	4.413 ± 0.02404^{cd}
SH 13 (15 g)	UV 15	7.623 ± 0.01202^{f}	5.553 ± 0.02028^{c}	3.100 ± 0.04359^{a}
SH 13 (15 h)	UV 15	9.406 ± 0.00333^{q}	8.216 ± 0.00667^{m}	3.676 ± 0.02404^{b}
SH 13 (15 i)	UV 15	9.923 ± 0.00882^{r}	7.986 ± 0.06766^{l}	5.240 ± 0.02082^{fg}
SH 13 (15 j)	UV 15	8.743 ± 0.00882^{m}	8.173 ± 0.02728^{m}	6.290 ± 0.04163^{k}
SH 13 (30 a)	UV 30	8.706 ± 0.01453^{m}	7.683 ± 0.01764^{jk}	6.170 ± 0.03055^{jk}
SH 13 (30 b)	UV 30	14.427 ± 0.04978^{s}	14.167 ± 0.03528^{p}	14.013 ± 0.05044^{n}
SH 13 (30 c)	UV 30	9.123 ± 0.05239^{p}	6.250 ± 0.02309^{e}	4.793 ± 0.01667^{e}
SH 13 (30 d)	UV 30	8.280 ± 0.02082^{jk}	5.890 ± 0.04163^{d}	4.450 ± 0.02309^{cde}
SH 13 (30 e)	UV 30	7.690 ± 0.03055^{fg}	7.240 ± 0.01528^{i}	6.130 ± 0.05292^{jk}
SH 13 (30 f)	UV 30	7.306 ± 0.02603^{e}	6.750 ± 0.01528^{g}	5.573 ± 0.02728^{ghi}
SH 13 (30 g)	UV 30	8.750 ± 0.02309^{m}	5.326 ± 0.02186^{b}	4.703 ± 0.02906^{de}
SH 13 (30 h)	UV 30	9.183 ± 0.06009^{p}	6.290 ± 0.01000^{e}	4.380 ± 0.01732^{cd}
SH 13 (30 i)	UV 30	9.140 ± 0.01000^{p}	8.786 ± 0.02028^{o}	5.366 ± 0.63677^{fg}
SH 13 (30 j)	UV 30	8.920 ± 0.01528^{o}	8.630 ± 0.16042^{n}	5.586 ± 0.04485^{ghi}
SH 13 (30 k)	UV 30	8.943 ± 0.01202^{o}	7.627 ± 0.04055^{jk}	6.893 ± 0.03844^{l}
SH 13 (45 a)	UV 45	7.706 ± 0.01453^{g}	6.283 ± 0.01764^{e}	5.410 ± 0.02309^{fgh}
SH 13 (45 b)	UV 45	8.220 ± 0.05132^{ij}	7.570 ± 0.02000^{j}	6.826 ± 0.03283^{l}
SH 13 (45 c)	UV 45	7.843 ± 0.02404^{h}	7.716 ± 0.01764^{k}	4.513 ± 0.02028^{cde}
SH 13 (45 d)	UV 45	7.210 ± 0.01155^{d}	6.876 ± 0.04807^{h}	5.870 ± 0.03055^{ij}
SH 13 (45 e)	UV 45	8.363 ± 0.01856^{l}	6.633 ± 0.01202^{f}	5.766 ± 0.02603^{hi}
SH 13 (45 f)	UV 45	6.520 ± 0.01000^{b}	6.223 ± 0.02404^{e}	4.350 ± 0.02309^{cd}
SH 13 (45 g)	UV 45	6.616 ± 0.02028^{c}	6.166 ± 0.03844^{e}	5.227 ± 0.04485^{fg}
SH 13 (45 h)	UV 45	7.720 ± 0.01528^{g}	5.193 ± 0.02028^{a}	3.306 ± 0.00882^{a}
SH 13 (45 i)	UV 45	7.300 ± 0.02000^{e}	7.143 ± 0.03528^{k}	6.776 ± 0.02404^{l}
SH 13 (45 j)	UV 45	7.206 ± 0.01667^{d}	7.590 ± 0.02517^{l}	6.720 ± 0.01732^{l}

Screening of *Aspergillus terreus* and their mutants for Cr tolerance: Aspergillus terreus SH 13 was subjected to UV (260nm) exposure for 15 to 45 min time intervals. Based on morphological features of their colonies, 29 variants were recovered which were then exposed to varying levels of Cr^{+6} in Czapek dox broth. (Table 1). Most of the UV irradiated variants of SH 13 like SH 13 (15c), SH 13 (15e), SH 13 (15f), SH 13 (30a), SH 13 (30g), SH 13 (30h), SH 13 (45a), SH 13 (45f), SH 13 (45g) and SH 13 (45h) lost its ability to resist the exceeding level of heavy metal Cr. All the strains showed minimal growth in Czapek dox broth at 900 ppm of Cr. Some of the UV irradiated variants of *Aspergillus terreus* remained unaffected and showed no visible change in their growth pattern under stress. Surprisingly among all the 31 variants, SH 13 (30b) showed highest growth in the presence of chromate stress. Growth of this strain was better than the wild type strain even in presence of 900 ppm of chromate. This mutant was selected for comparison with the wild type strain. Morphology of fungal colony on PDA and microscopic characteristic of SH 13 (30b) are shown in Figure 3.

Quantification of phytohormones and metabolites in fungal culture filtrate (FCF) of *Aspergillus terreus* under stress: Fungal strain *Aspergillus terreus* produced significant amounts of IAA both under normal and stressful conditions (Fig. 4A). An increase in the IAA production was observed with the increase in heavy metal stress. In comparison to the wild type strain, the UV irradiated variant SH 13 (30b) produced significantly greater quantity of IAA. The mutant also continued to release higher quantities of IAA with increase in the amount of chromate in the medium. Production of flavonoids by both the wild strains and UV irradiated strain showed gradual increase with the increasing heavy metal stress. In the absence of chromate stress, both the wild and mutant strains of *Aspergillus terreus* produced lesser flavonoids in the Czapek dox broth, but the pattern changed when the strains was allowed to grow under chromate stress. Under Cr stress, the UV irradiated strain produced significant amount of flavonoids in comparison to the wild type (Fig. 4B). The wild strains produced higher level of salicylic acid in comparison to the mutant. However, the pattern changed drastically as Cr level

exceeds 300 ppm. At 600 ppm, the UV irradiated strain (SH 13 30b) produced greater amount of salicylic acid up to 16.72% in comparison to the wild type (Fig. 4C). The maximum amount of salicylic acid (208.88µg/ml) was produced by the mutant strains exposed to 900 ppm of chromate. The production of salicylic acid in the mutant strain tends to increase at elevating levels of chromate (Fig. 4C). The pattern of total phenolics in the fungal strains was almost similar to salicylic acid. Under normal conditions, both the wild and mutant strain was able to produce almost equal amount of phenolic content however, the pattern changed when the strains were exposed to higher level of chromate stress. The wild strain of SH 13 was able to produce 451 µg/ml of phenolics, while the UV irradiated produced 571 µg/ml in Czapek broth supplemented with 900 ppm of Cr^{+6} (Fig. 4D).

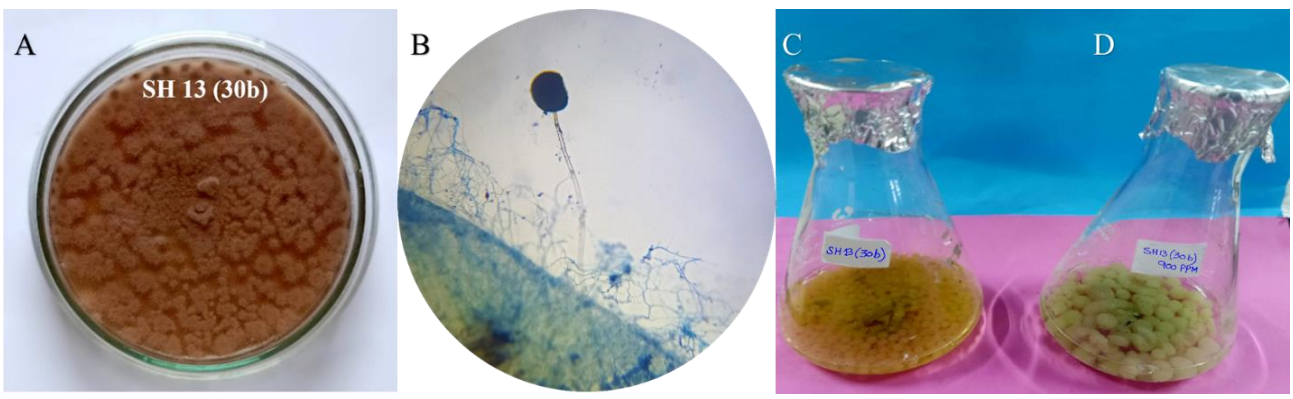

Fig. 3. Characterization of *Aspergillus terreus* SH 13 exposed to UV for 30 min (SH 13 (30b); **A)** Colony morphology of Aspergillus terreus on PDA, **B)** Light microscopy of the lactophenol cotton blue stained hyphae, and **C)** growth pattern in Czapek broth alone or **D)** supplemented with 900 ppm of chromate.

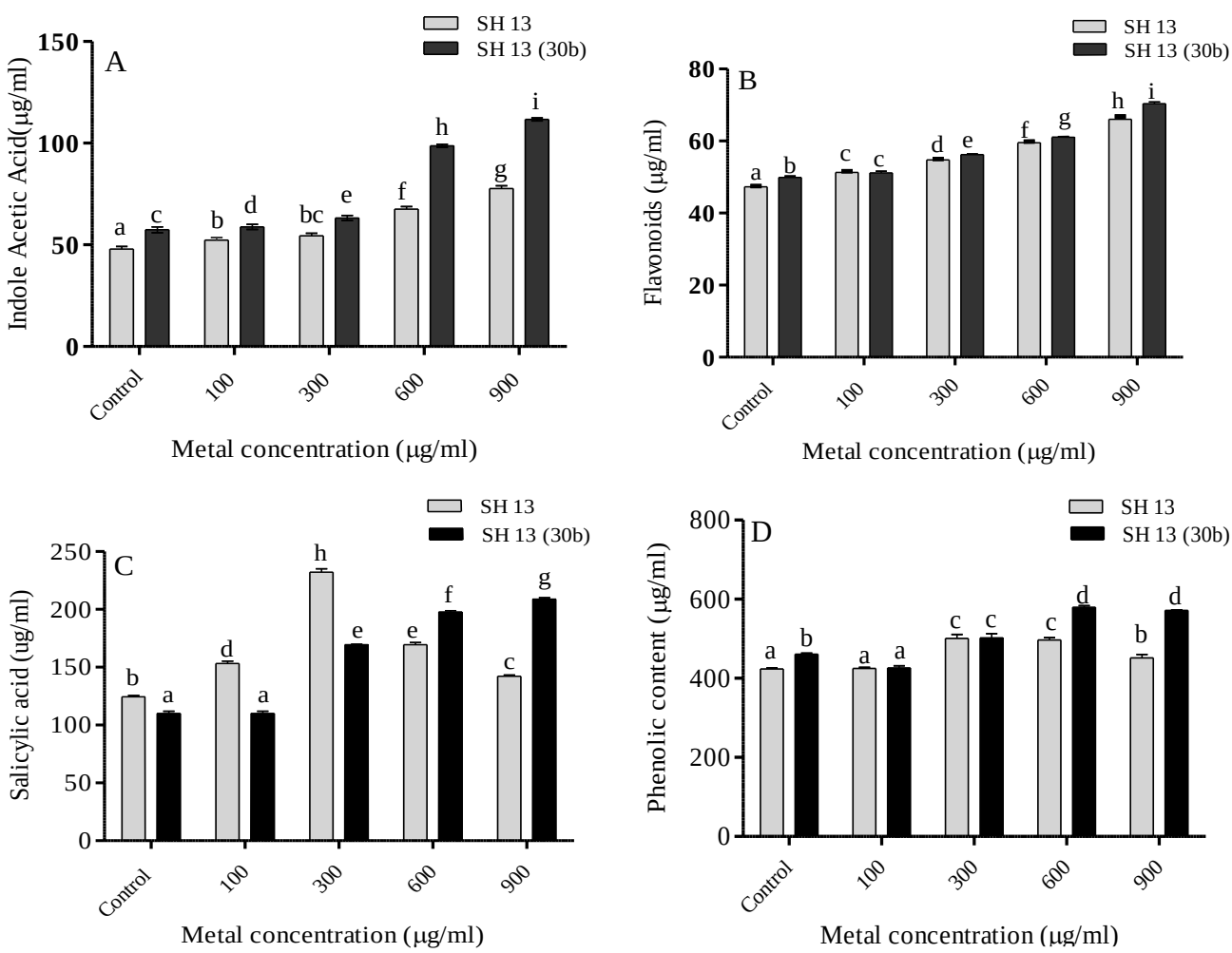

Fig. 4. Production of **A**) IAA, **B)** Salicylic acid **C)** Flavonoids and **D)** Phenols by fungal strain SH 13 and its mutant (SH 13 30b) under varying concentration of chromate stress. Data shown are mean ± SE of 3 replicates and labels on bars denotes significance among treatments ($p<0.05$).

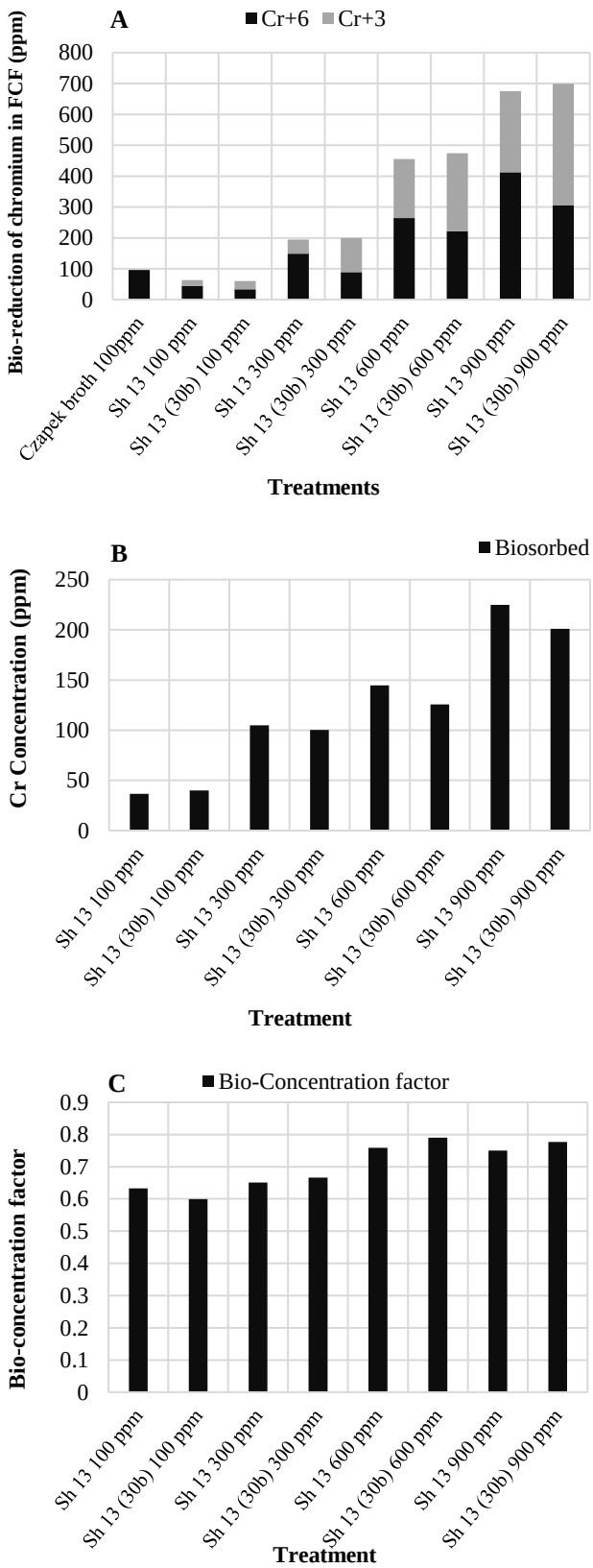

Fig. 5. Impact of *Aspergillus terreus* (SH 13) and its mutant SH 13 (30b) on **A)** Bio-reduction of Cr from hexavalent to the trivalent form in FCF, **B)** Biosorption and **C)** Bio-concentration factor. Data shown are mean replicates with ± SE ($p<0.05$).

Bio-reduction of hexavalent chromium to trivalent form: The efficacy of the UV-C irradiated strain to bio transforms the hexavalent form of chromium to trivalent was considerably higher in comparison to wild type fungal strain (non- radiated). After 7 days of incubation in czapek dox broth containing 100 ppm chromate, both the UV variant and the wild type strain the UV-C irradiated strain bio-transformed half of the total chromium in FCF to their trivalent form (Fig. 5A). Mutant strain SH 13 (30b) showed least absorption of Cr in comparison to the wild strain. At 900 ppm, wild Aspergillus terreus biosorbed 24.97% of heavy metal Cr while mutant SH 13 (30b) biosorbed lesser amount of Cr which is 22.34% of total supplemented Cr (Fig. 5B).

Chromium stress mitigation in *B. napus*: Different levels of chromate stress (25 and 50 ppm) were applied in the soil used to grow B. napus seedlings. Both the selected levels of chromate stress retarded different growth attributes of *B. napus* seedlings (Fig. 6A, 6B). Root and shoot lengths of the chromate (50ppm) exposed seedlings were reduced to 9.334% and 21.68% of the control seedlings respectively. Fresh weight of the seedlings exposed to 25 ppm and 50 ppm was reduced to 20.41% and 26.46% of the control respectively. Chlorophyll a content was also reduced in chromate stressed seedlings than the control. Both the wild and mutant strains significantly promoted seedlings growth attributes of control and chromate stress seedlings. However, the UV irradiated variant was more beneficial for the growth of B. napus seedlings. Inoculation of seedlings with SH 13 promoted shoot and root growth by 79.36% and 62.4% than the control seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate; their shoot growth was only 51.78% and 47.86% of the SH 13 colonized seedlings exposed to these levels of chromate respectively. The UV irradiated variant 30b promoted shoot length and root length by 246.55% and 8.9% than the control respectively. Exposure of 30b associated seedlings to the mentioned levels of chromate reduced root and shoot lengths but their growth was still much higher than the non-endophyte counterparts. Inoculation of the seedlings with wild SH 13 increased the fresh weight of seedlings by 228.29% than the control seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate; their total weight was only 41.36% and 60.56% of the SH 13 colonized seedlings exposed to these levels of chromate respectively. The UV irradiated endophytic fungi increased the total fresh weight of the seedlings by 1718% almost 17 folds than the control respectively (Fig. 6C).

Seedlings inoculated with SH 13 increased the chlorophyll a and b content by 61.77% and 31.71% than the control seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate; their Chlorophyll a content was only 63% and 44.09% of the SH 13 colonized seedlings exposed to these levels of chromate respectively. Similarly, the chlorophyll b content in the non-endophyte seedlings was only 75.36% and 64.97 % of the SH 13 colonized seedlings exposed to these levels of chromate respectively. At elevated level of heavy metal stress i.e., 50 ppm of Cr, UV irradiated variant 30b promoted the chlorophyll a and b contents by 166.3% and 218.3% of the control seedlings exposed to same level of chromate stress (Fig. 6D, 6E).

Inoculation of seedlings with SH 13 promoted the total chlorophyll content by 51.65% than the control seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate; their total chlorophyll content was only 66.18% and 48.37% of the SH 13 colonized seedlings exposed to these levels of chromate respectively. Total chlorophyll content was higher in 30b inoculated seedlings than the wild inoculated and non-endophytic seedling. The UV irradiated variant 30b showed increase in the total chlorophyll content by 59.52% than the control respectively. Exposure of 30b associated seedlings to the mentioned levels of chromate showed no effect on the total chlorophyll content. Highest amount of total chlorophyll content was observed in seedlings inoculated with 30b under 50ppm of Cr stress (Fig. 6F).

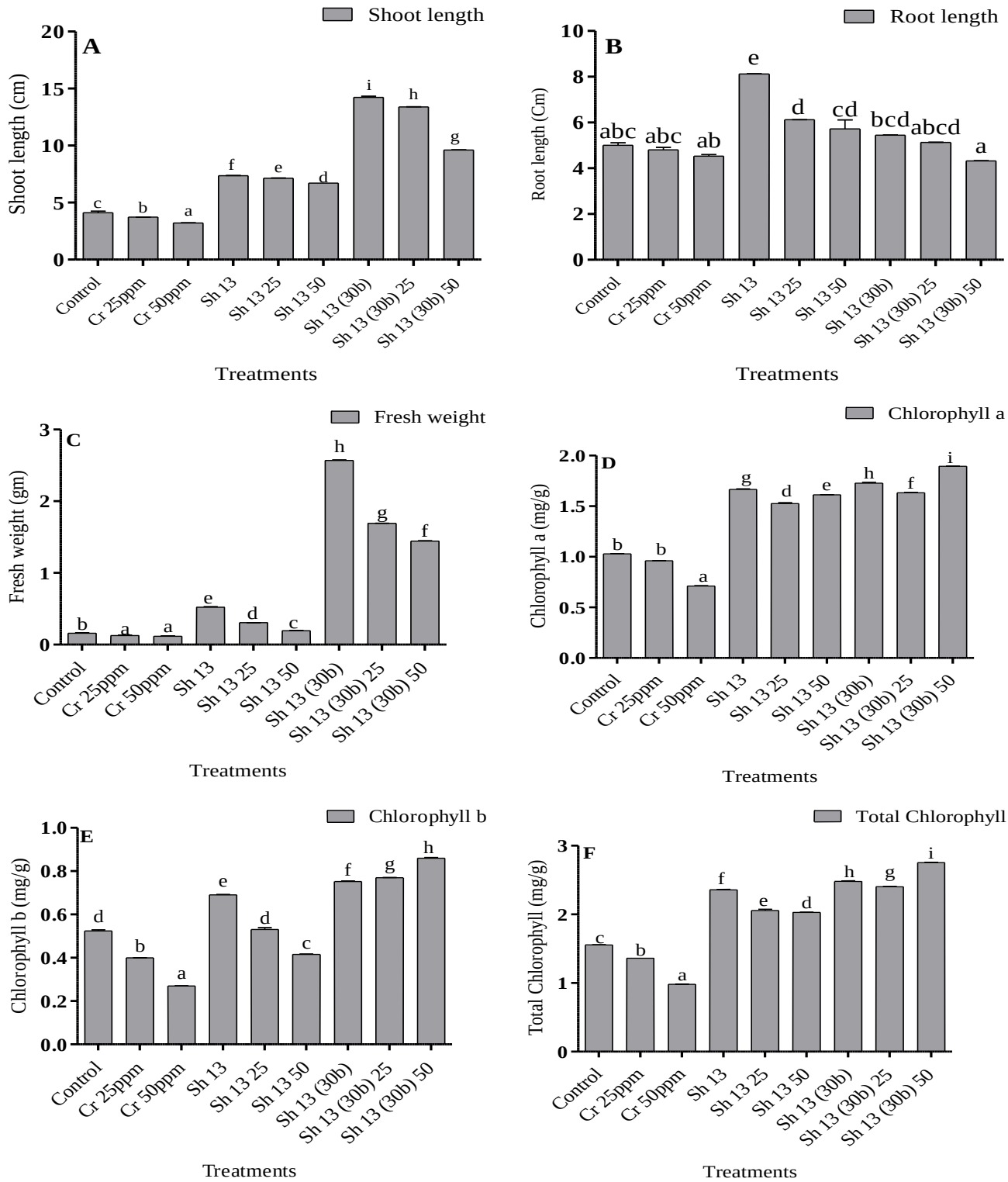

Fig. **6**. Effect of Endophytic fungi *Aspergillus terreus* (wild and UV irradiated) on **A)** Shoot length, **B)** Root length, **C)** fresh weight, **D)** chlorophyll A, **E)** chlorophyll B and **F)** total chlorophyll spiked with or without Cr^{+6}. Values are means of 3 replicates with ±SE (Duncan test $p<0.05$).

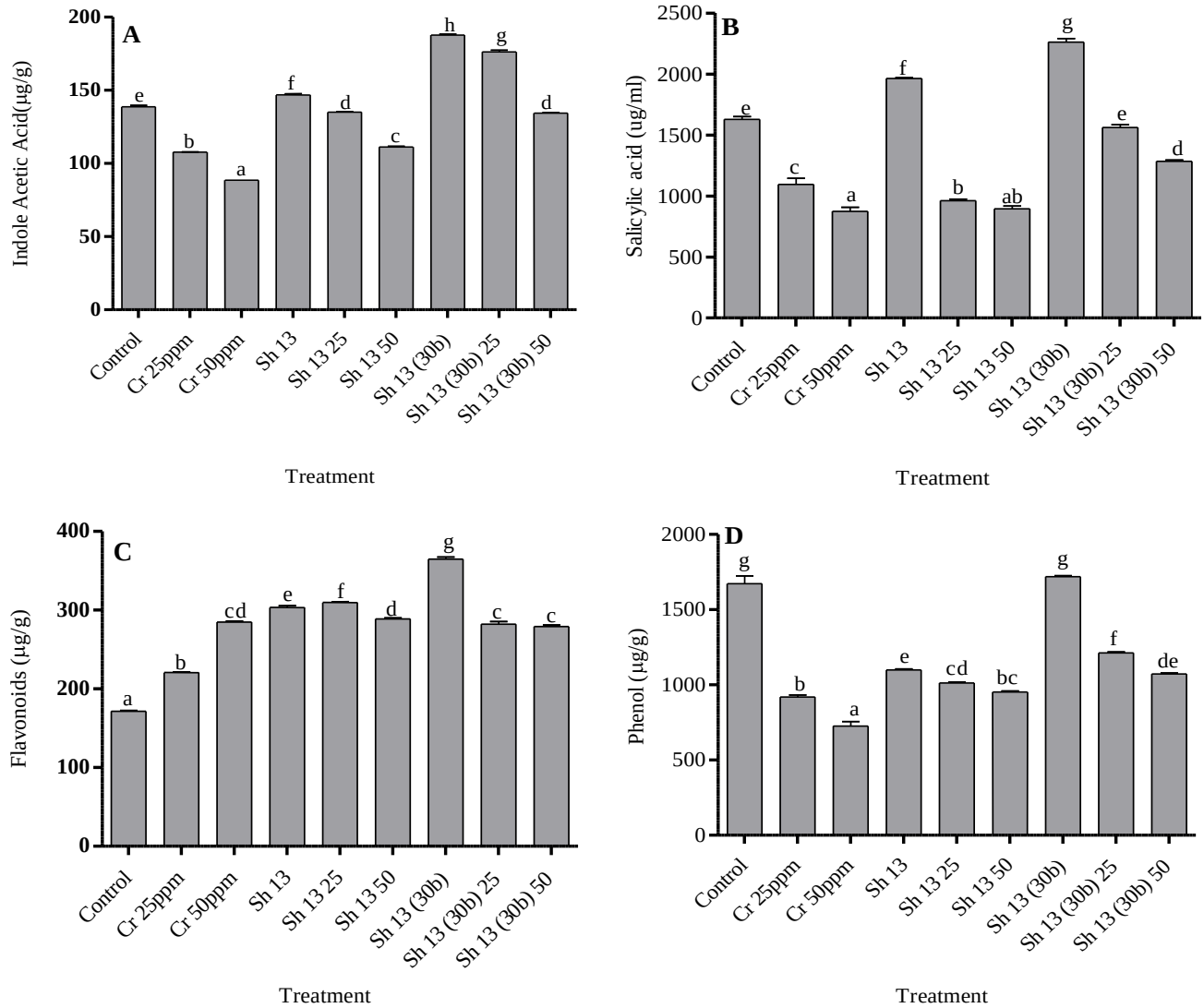

Fig. 7. Effect of heavy metal (Cr^{+6}) and *A. terreus* inoculation on **A)** IAA, **B)** Salicylic acid **C)** Flavonoids, and **D)** Phenol contents in *Brassica napus* seedlings. Data shown are mean ± SE of 3 replicates and labels on bars denotes significance among treatments ($p<0.05$).

Effect of wild and UVC irradiated *Aspergillus terreus* on the plant metabolites under heavy metal stress: Different levels of chromate stress (25 and 50 ppm) adversely affected the production of plant metabolites. Levels of IAA in shoot of *B. napus* under Cr^{+6} stress was declined with the increasing Cr^{+6} stress. The level of IAA was reduced from 138.7 µg/gm to 88.5µg/gm with the increasing chromium stress from 0 to 50 ppm. However, inoculation of the seedlings with wild SH 13 and UV-C irradiated SH 13 benefited the plant by improving the production of IAA. Levels of IAA in the chromate (50ppm) exposed seedlings were reduced to 63.79% of the control seedlings respectively. Both the wild and mutant strains significantly promoted the efficacy of seedlings to produce IAA in the control and chromate stressed seedlings. However, the UV irradiated variant 30b was more beneficial for the production of IAA in B. napus seedlings. Inoculation of seedlings with SH 13 promoted the production of IAA by 5.79% than the control seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate, their IAA content was only 79.72% and 79.53 % of the SH 13 colonized seedlings exposed to these levels of chromate respectively. The UV irradiated variant 30b promoted the production of IAA by 35.35% than the control respectively. Exposure of 30b associated seedlings to the mentioned levels of chromate reduced the production of IAA but their production rate was still much higher than the non-endophyte counterparts (Fig. 7A). The net production of salicylic acid was enhanced when seedlings were inoculated with wild and UV-C irradiated SH 13 (30b). Levels of salicylic acid in the chromate (25 and 50ppm) exposed seedlings were reduced to 67.25% and 53.61% of the control seedlings respectively. The wild strains adversely effected the production of Salicylic acid in the seedlings however the mutant strains significantly promoted the efficacy of seedlings to produce salicylic acid in the control and chromate stressed seedlings. Inoculation of seedlings with SH 13 promoted the production of salicylic acid by 20.60% than the control seedlings. The UV irradiated variant 30b promoted the production of salicylic acid by 38.88% than the control respectively. Similarly, when non-endophytic seedlings were exposed to 25 and 50 ppm chromate, their efficacy to produce salicylic acid was only 70.12% and 67.99% of the mutant 30b colonized seedlings exposed to these levels of chromate respectively (Fig. 7B).

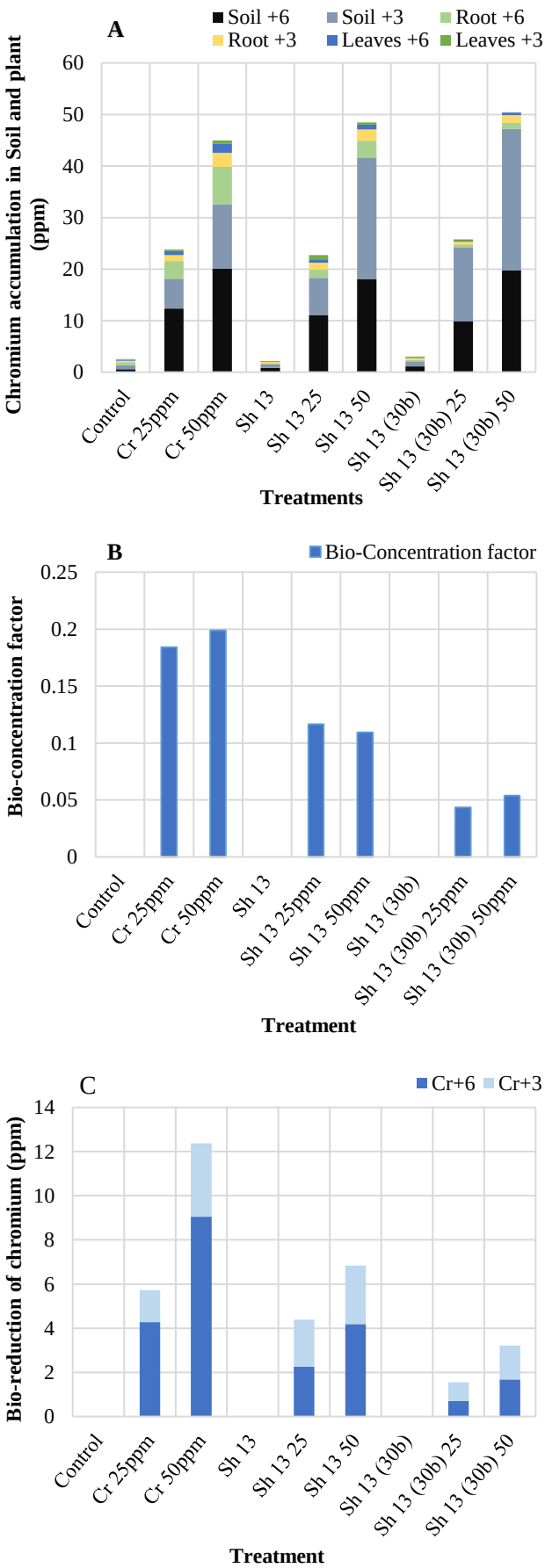

Fig. 8. Effect of *Aspergillus terreus* on **A)** Chromium in soil and their translocation to various parts **B)** Bio-concentration factor and **C)** Bio-reduction of Cr.

Production of flavonoids in the seedlings increased with increasing chromium stress in dose dependent manner. The amount of flavonoids in seedling inoculated with SH 13 and UV-C irradiated SH 13 (30b) showed great diversity. Seedlings inoculated with UV-C irradiated SH 13 produced greater quantities of flavonoids in comparison to seedling supplemented with wild SH 13 (Fig. 7C). Seedlings inoculated with wild SH 13 strain produced higher amount of flavonoids than control. However, the net production of flavonoids was enhanced when it was inoculated with UV-C irradiated SH 13 (30b). UV irradiated variant was more beneficial for the production of flavonoids in B. napus seedlings. The UV irradiated variant 30b promoted the production of flavonoids by 112.86% than the control respectively. Similarly, when non-endophytic seedlings were exposed to 25 chromates, their efficacy to produce flavonoids was 78.13% of mutant 30b colonized seedlings exposed to same level of chromate (Fig. 7C). The amounts of total phenolics were fairly enhanced in seedlings inoculated with UV-C irradiated SH 13 in comparison to the control and wild SH 13 inoculated seedlings. The UV irradiated variant was more beneficial for the production of total phenolics in B. napus seedlings. Inoculation of seedlings with mutant 30b promoted the production of total phenolics by 2.78% than the control seedlings. Exposure of 30b associated seedlings to the mentioned levels of chromate reduced the production of total phenolics but their production rate was still higher than the non-endophyte counterparts (Fig. 7D).

Chromium in soil and seedlings: Concentration of Cr-III and Cr-VI was found in the soil and different parts of the seedlings using atomic absorption spectrometer. In the non-endophytic seedlings, 72.44% and 65.2% of the chromium remained in soil supplemented with 25 and 50 ppm respectively. Remaining Cr was absorbed by the seedlings which was distributed in their different parts (Fig. 8A). In the soil supplied with 25 and 50 ppm Cr, 31.75% and 38.34% of the unabsorbed Cr was reduced to Cr-III respectively. Most of the Cr (18.4% of 25 ppm and 19.9% of 50 ppm) absorbed by the seedlings was retained in their roots. In seedlings treated with 25 ppm chromate, 25% and 26.66% of the Cr was reduced to Cr-III in the root and leaf respectively. Inoculation with the wild type and UV variant fungal endophytes significantly reduced Cr uptake by the seedlings. It was noticed that the SH 13 colonized seedlings absorbed only 17.55% and 13.66% of the 25 and 50 ppm chromium added in the soil respectively. In SH 13 colonized seedlings, most of the absorbed Cr was represented by Cr-VI. The uptake of Cr was noticeably decreased when the seedlings were supplemented with UV irradiated SH 13 30b. In seedlings supplemented with mutant SH 13 30b most of the Cr, i.e., 96.96% and 94.4% of 25 and 50 ppm retained in the soil while least absorption by the seedlings was observed which was 3.04% and 5.6% of 25 and 50 ppm respectively (Fig. 8A). In seedlings treated with 25 ppm chromate in presence of SH 13 30b, 49.83% and 63.31% of the absorbed Cr was reduced to Cr-III in the root and leaf respectively.

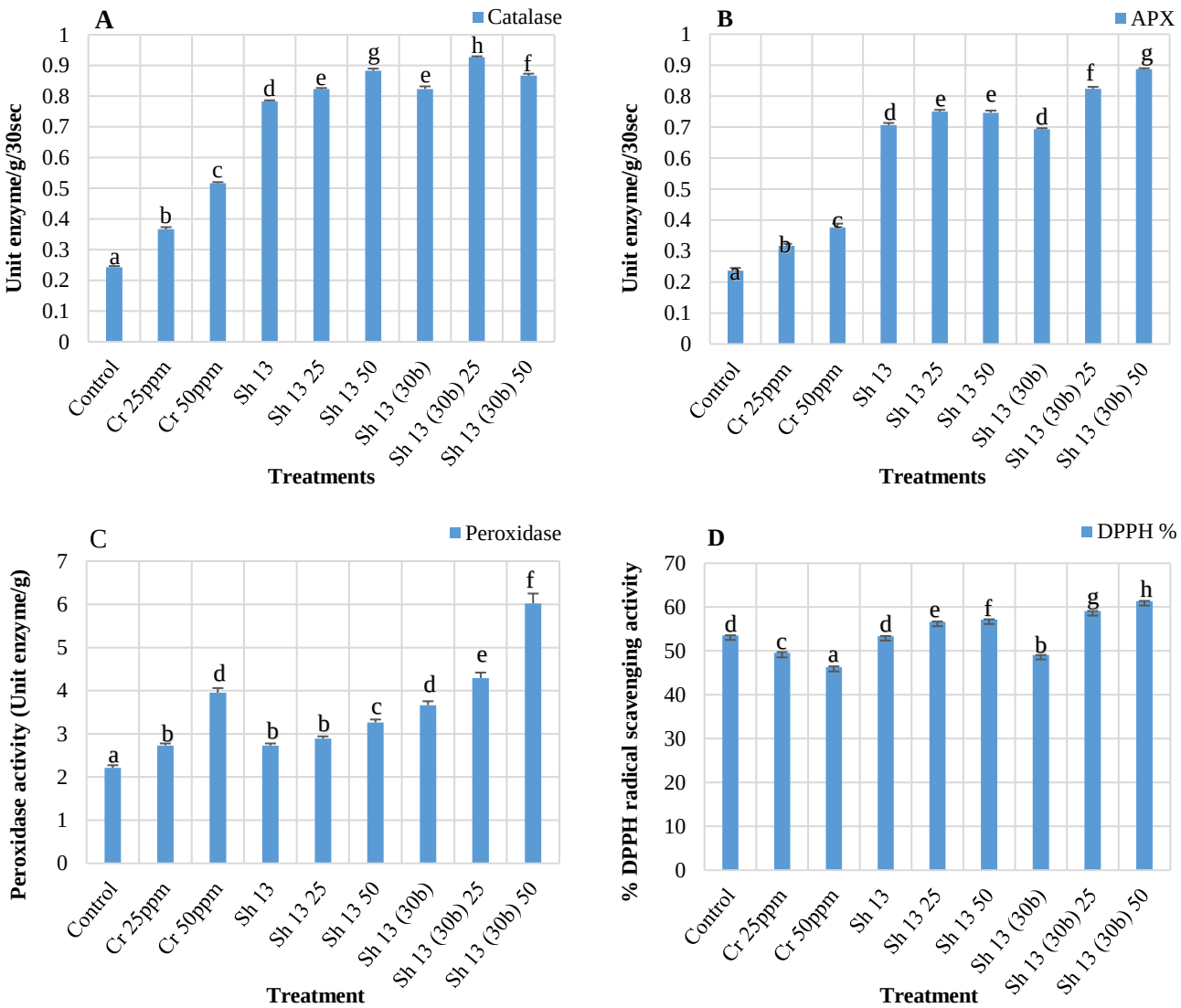

Fig. 9. Effect of Cr^{+6} stress and SH 13 inoculation on **A)** Catalase, **B)** Ascorbate peroxidase, **C)** Peroxidase and **D)** DPPH in host plants. Data represent mean with SE± and letters represents significant difference ($p<0.05$).

Bio-concentration factor: Seedlings of *B. napus* grown in the presence and absence of UV irradiated and wild SH 13 strains were assessed for the absorption and bio-accumulation of Cr. Bioaccumulation of Cr in the seedlings increased with the increasing amount of Cr. Non-endophytic seedlings have accumulated 27.68% and 34.8% of Cr under 25 and 50 ppm stress. Seedlings supplemented with wild SH 13 accumulated 26.76% and 16.72% of Cr in comparison to non-endophytic seedlings at 25 ppm and 50 ppm. However, the bioaccumulation was reduced up to a remarkable extent of 3.04% and 5.6% of the control in the seedling supplemented with UV irradiated SH 13 (30b) (Fig. 8C).

The biotransformation of Cr from its toxic to least toxic state was identified using the BCR extraction method. The fungal strain SH 13 not only allowed the least absorption, but also showed its significant abilities in transforming the Cr from its highly toxic (Cr^{+6}) state to the least toxic (Cr^{+3}) one. Plant inoculated with UV irradiated SH 13 strains showed highest biotransformation of 53.87% and 47.99% at 25ppm and 50 ppm respectively (Fig. 8C).

***A. terreus* regulates antioxidant enzyme system in *B. napus*:** An evident rise in CAT, APX, and peroxidase activity was noted as the Cr stress increased (Fig. 9). The exposure of chromate (25 and 50 ppm) resulted in a significant increase in catalase activity, reaching 50.71% and 112.37% of the control seedlings, respectively. Both the wild and mutant strains exhibited a robust enhancement in CAT activity under both normal and chromate-stressed conditions. Particularly, the UV-irradiated variant 30b demonstrated remarkable efficiency in inducing higher CAT activity in B. napus seedlings. On the other hand, non-endophyte seedlings exposed to 25 and 50 ppm chromate exhibited only 44.54% and 58.49% of the catalase activity observed in SH 13 colonized seedlings exposed to the same levels of chromate, respectively. The UV irradiated variant 30b increased catalase activity by 238.38% than the control respectively. Similarly, when non-endophytic seedlings were exposed to 25 and 50 ppm chromate, their overall catalase activity was only 39.57% and 59.61% of the mutant 30b colonized seedlings exposed to these levels of chromate respectively (Fig. 9A). Higher ascorbate peroxidase activity was observed in endophytic colonized seedlings in comparison to non-endophytic seedlings. Ascorbate peroxidase activity in the

chromate (25 and 50ppm) exposed seedlings increased up to 33.79% and 59.14% in comparison to control seedlings. The wild strains promoted APX activity in the seedlings however the mutant strains vigorously increased APX activity in the control and chromate stressed seedlings. UV irradiated variant 30b was more beneficial to induce higher APX activity in B. napus seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate, observed APX activity was only 42.22% and 50.44% of the SH 13 colonized seedlings exposed to these levels of chromate respectively. The UV irradiated variant 30b increased APX activity by 192.9% than the control respectively. Similarly, when non-endophytic seedlings were exposed to 25 and 50 ppm chromate, their overall APX activity was only 38.46% and 42.48% of the mutant 30b colonized seedlings exposed to these levels of chromate respectively (Fig. 9B). Increasing heavy metal stress resulted in higher peroxidase activity in the seedlings. Peroxidase activity in the chromate (25 and 50ppm) exposed seedlings increased up to 23.50% and 78.9% in comparison to control seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate, observed peroxidase activity was 63.55% and 65.68 % of mutant 30b colonized seedlings exposed to these levels of chromate respectively (Fig. 9C). A gradual decrease in the DDPH activity was observed with increasing heavy metal stress in the control seedling. DPPH activity in the chromate (25 and 50ppm) exposed seedlings decreased up to 7.43% and 13.46% of the control seedlings respectively. In heavy metal stress UV irradiated variant 30b was more beneficial to induce higher DPPH activity in B. napus seedlings. When the non-endophyte seedlings were exposed to 25 and 50 ppm chromate, observed DPPH activity was only 87.47% and 81.04% of the SH 13 colonized seedlings exposed to these levels of chromate respectively. DPPH activity of non-endophytic seedlings exposed to 25 and 50 ppm chromate was only 83.88% and 75.50% of the mutant 30b colonized seedlings exposed to these levels of chromate respectively (Fig. 9D).

3, 3-Diaminobenzidine (DAB) stain assay: The production of H_2O_2 showed a clear positive relationship with the increasing concentration of Cr^{+6}. Plants exposed to higher concentrations of Cr^{+6} exhibited denser brown spots spread over a larger area, in contrast to plants supplemented with lower levels of chromate (Fig. 10). Control leaves displayed no visible spots. However, the inoculation with the SH 13 strain significantly reduced the production of H_2O_2. Moreover, when the plants were exposed to both Cr^{+6} and UV-irradiated 30b, the leaf tissues showed no spots upon exposure to DAB stain, indicating the lowest production of H2O2 in these tissues.

Root colonization: Root colonization potential of the endophytic fungi was assayed by observing the lactophenol cotton blue stained sections under a light microscope. It was observed that endophytic fungi have successfully colonized the plant roots when supplemented with endophytic fungal biomasses (Fig. 11).

Discussion

Ultraviolet radiation as physical agent is considered as an efficient method to improve most of the microbial strains (Nicolas-Santiago *et al.,* 2006; Huang *et al.,* 2019; Jan *et al*., 2022). Ultraviolet radiations were used to initiate mutagenesis in *A. terreus* to provide an appropriate mutant strain that can resist the hazardous effect of Cr^{+6} and promote plant growth. Among 29 variants retrieved after UV irradiation, *A. terreus* (SH 13 30b) not only showed maximal resistance to increasing concentrations of Cr^{+6} but also showed promising growth promoting abilities. The noticeable fact was UV irradiated *A. terreus* secreted high IAA, SA, flavonoids, sugar and phenol to help them resist the stressful conditions. Compared to non-UV irradiated *A. terreus,* the overall production of phytohormones by the UV irradiated *A. terreus* was considerably high. Also, the fungal culture sustained greater phytohormones to biomass ratio in the presence of chromium stress. The greater phytohormones to biomass ratio demonstrates its adaptability to the stressed and chronic conditions (Yang *et al.,* 2024; Shahbaz *et al.,* 2019). One of the important strategies of endophytes to adapt HMs stress is to decrease its surface area. UV irradiated A. terreus showed promising Cr resisting capabilities by effectively decreasing the surface area which enable them divide and reduce the stress. This decrease in surface area not only allows them to divide and lessen the stress, but also helps in bio-transforming the heavy metal to least toxic state in their close vicinity very effectively (Elmeihy *et al.,* 2021; Wang *et al.,* 2020).

Brassica seedlings exposed to different concentrations of Cr stress resulted in severe reduction of agronomic attributes, like root and shoot lengths, fresh and dry weights, chlorophyll and carotenoid contents. However, co-cultivation of the seedlings with UV irradiated *A. terreus* improved the agronomic features of the host. In addition, a substantial proliferation in plant's IAA, flavonoids and SA was recorded. These hormonal changes plant are one of the promising approaches for plants protection against the variety of stresses including HM's stress (Egamberdieva *et al.,* 2018). In present finding, UV irradiated *A. terreus* produced maximal amount of IAA, SA and flavonoids in order to mitigate the HMs stress and also triggered the host plant to produce increasing amounts under increasing Cr stress. In comparison to UV irradiated *A. terreus*, non-irradiated one produced low amounts of IAA, SA and flavonoids with low growth rate. Increasing amounts of IAA helps the plant to resist the heavy metal stress by disturbing the activity of DELLA protein or through regulation of enzymes which usually detoxify ROS (Paponov *et al.,* 2008). Besides, higher amounts of endogenous SA production is helpful in resisting the heavy metal stress as they generate defense related responses in the course of the abiotic stress (Hayat *et al.,* 2010). Flavonoids are also known to protect the plant against variety of abiotic stresses as well as increases plant microbes interaction (Hussain *et al.,* 2018; Xiong *et al.,* 2022). The brassica plants co-cultivated with UV irradiated *A. terreus* under heavy metal stress accumulated higher amounts of flavonoids to resist the detrimental effects of HMs (Vacheron *et al.,* 2013). The synthesis of phenolics by the brassica plants increased with increasing heavy metal stress. Production of higher amounts of phenolics might resist metal and oxidative stress by quenching the ROS and chelation of metals (Hayat *et al.,* 2010).

In plants ROS are produced and eliminated in an even manner in order to retain the usual metabolic activities of the plant. However, this balance is disrupted

whenever a plant is exposed to abiotic stress. Abiotic stress results in significant production of ROS thus disrupting the cell viability and yield a noticeable oxidative damage. High ROS accumulation results in leaking of the useful electrolytes out of the cell thus making the host more susceptible to apoptosis (Wu *et al.*, 2017). UV irradiated *Aspergillus terreus* has the exceptional ability to avoid oxidative stress by improving the synthesis of enzymatic antioxidants like APX, CAT, peroxidase and DPPH. Thus resulting in higher ROS scavenging and least accretion of ROS in the plants, which not only maintains the normal growth attributes of the plant during Abiotic stress but also results in visible growth improvement (Santander *et al.*, 2017).

Bio reduction of HM's from toxic to least toxic state is considered as one of the efficient way in order to decrease toxicity. AAS data reveals that in the presence of UV irradiated *Aspergillus terreus*, half of the Cr^{+6} was transformed to Cr^{+3} after one week of inoculation (Fan *et al.*, 2021). Bio-reduction of hexavalent Cr to trivalent Cr include two basic mechanisms. Either Cr^{+6} may reduce to Cr^{+3} by directly interacting with the walls of biomass or Cr^{+6} may bind to the amines of chitin and chitosan (positively charged) present in cell wall by possessing least reduction potential than that of Cr^{+6} (Ucun *et al.*, 2008; Shugaba *et al.*, 2012). In addition to the bio-reduction of the heavy metal, the normal growth pattern was rescued when the seedlings were supplemented with *Aspergillus terreus*. Growth induced by the foresaid endophytic fungi in both normal and HM stress suggest that heavy metal reclamation might not be the only way of phyto-stimulation (Shen *et al.*, 2013). Current study suggests that upon UV exposure, the effectiveness of *Aspergillus terreus* to escalate the agronomic features of the plants and to alleviate heavy metal stress by biotransformation and bio-reduction is considerably increased thus making them unreachable to roots (Hildebrandt *et al.*, 2007).

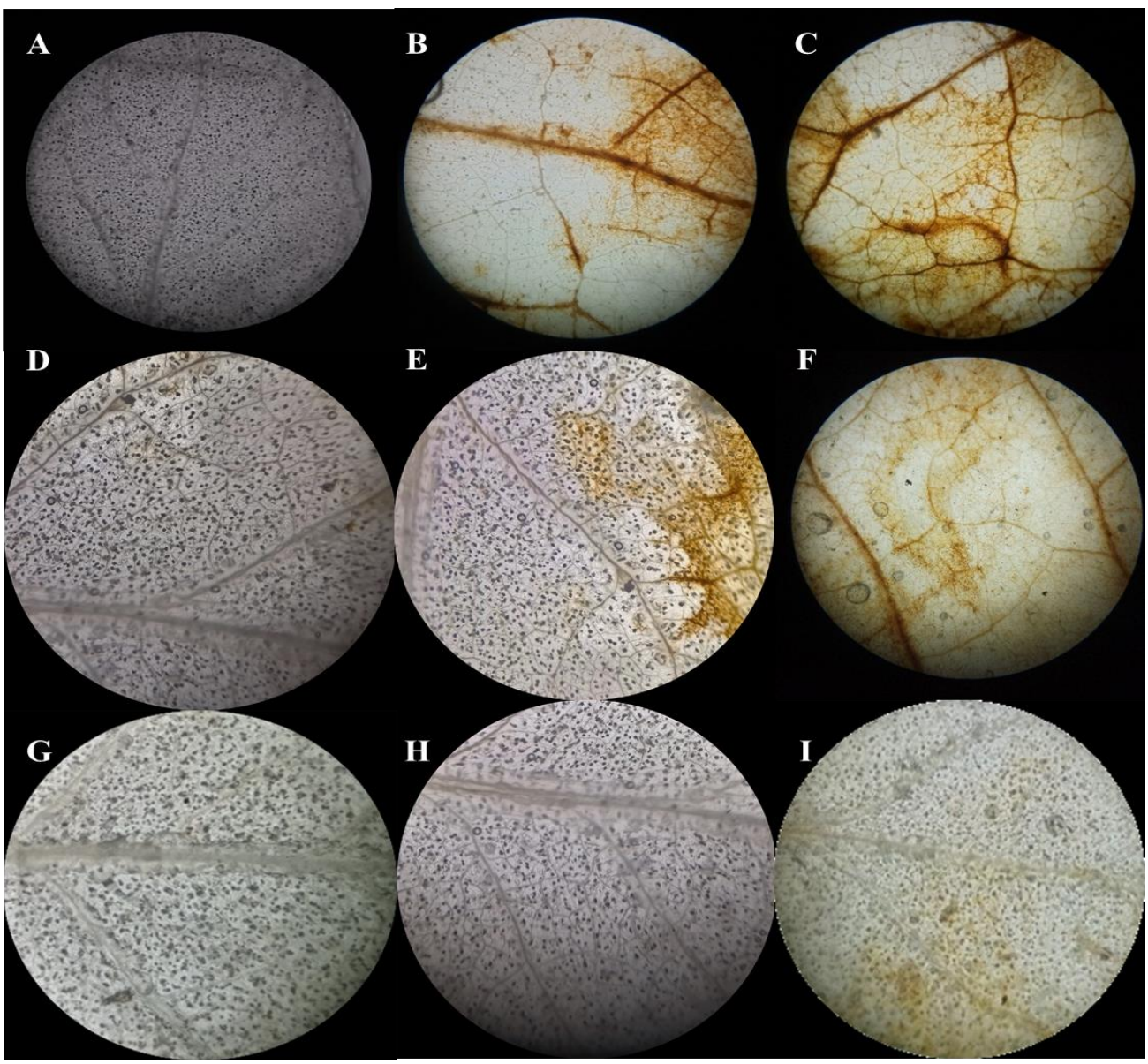

Fig. 10. ROS accumulation in SH 13 and UV irradiated SH 13 (30b) inoculated *Brassica napus* leaves in comparison to control under varying levels of Cr^{+6} stress. **A)** Control, **B)** 25 ppm chromate, **C)** 50 ppm chromate, **D)** SH 13, **E)** SH 13+ 25ppm chromate, **F)** SH 13 + 50 ppm chromate, **G)** SH 13 (30b), **H)** SH 13 (30b) + 25 ppm chromate and **I)** SH 13 (30b) + 50 ppm chromate. Fresh leaves of 16 days old seedlings were detached from the plants and were stained with DAB in order to visualize ROS.

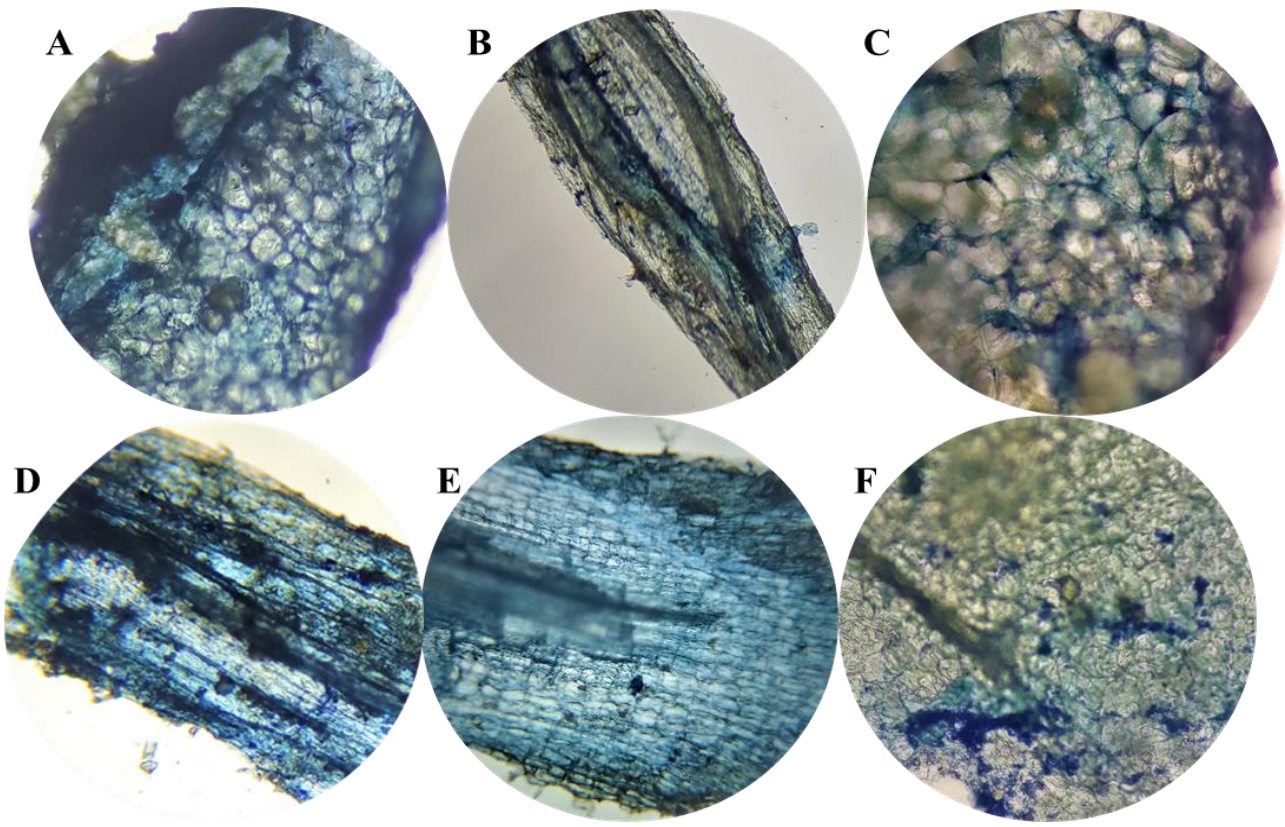

Fig. 11. Colonization of endophytic fungus *Aspergillus terreus* SH 13 (A, B, C) and its UV irradiated variant SH 13 (30b) (D, E, F) in the cortical region of *Brassica napus* root stained with lactophenol cotton blue dye after 15 days of successful colonization.

Conclusion

From current study it is concluded that upon UV exposure, the ability of *A. terreus* to alleviate heavy metal stress is considerably enhanced. The strain not only enhanced the antioxidant system of the host plant, but has also exceptional abilities to reduce Cr^{+6} to Cr^{+3}, thus there is subsequent low uptake and reduced toxicity in host plant species. Hence pre-exposing *A. terreus* to UV radiation may increase bio-remediating competences of the strain, which can be used as a proactive bio-fertilizer in Cr contaminated soils.

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