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FULVIC ACID MEDIATE CHROMIUM (Cr) STRESS IN MARIGOLD GENOTYPES (*Tagetes patula* L.) THROUGH LOWERING THE Cr UPTAKE AND IMPROVING PHYSIOLOGICAL PROCESSES

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Chromium (Cr) is one of the most important non-essential phytotoxic heavy metal for plants. The fulvic acid (FA) alleviate Cr toxicity in marigold genotypes. In this experiment, the soil contaminated with Cr concentration 8 mg kg^{-1} was used to grow the marigold genotypes Striped marvel and Inca. The FA 0, 2, 4, 6 and 8 mg L^{-1} were applied as foliar treatments. The result showed that the FA significantly improve the growth parameters, photosynthetic pigments, activities of antioxidative enzymes and reduced the production of reactive oxygen species (ROS) in both genotypes. The Cr in leaves and roots was observed minimum at FA @ 8 mg L^{-1} applied and maximum where no FA was applied in both marigold genotypes. The FA also reduced the translocation, bioaccumulation, and phytoextraction of Cr. The marigold genotype striped marvel was found tolerant genotype as compared to Inca. It is concluded that the application of FA is one of the best useful solutions for the mitigation of Cr toxicity in marigold genotypes. Thus, the marigold may be grown as an ornamental plant with the foliar application of FA may be used as a sustainable remediation strategy in Cr contaminated soils and also improved the aesthetic environment.
Keyword: Reactive oxygen species, antioxidant enzymes, translocation, bioaccumulation, phytoextraction.

INTRODUCTION

Today, the soil contaminated with heavy metals is one of the most critical global concern (Lwin *et al.,* 2018). This problem is constantly increased due to industrial development and urbanization (Yadav *et al.,* 2017; Naseem *et al.,* 2020). In developing countries like Pakistan, the industrial wastewater carrying a rich amount of heavy metals dumped on agricultural lands also pollutes our groundwater resources (Murtaza *et al.,* 2017; Hina *et al.,* 2019).

Chromium (Cr) is a hazardous, persistent and mutagenic heavy metal that deteriorates the environment. Improper disposal and management of wastewater from tanning, paint, electroplating industries are an important source of Cr pollution (Rahman and Singh, 2019). The wastewater disposal on lands and used for agricultural purposes contaminate our food chain (Alvarenga *et al.,* 2017; Riaz *et al.,* 2018). Cr entered into human beings via the food chain and causes cancer. It is a highly toxic non-essential heavy metal which decreases plant growth. The Cr VI is more toxic, hazardous, lethal as compare to Cr III. It is also frequently used by industries. The more the usage in industries more the concentration of Cr released in soils and water bodies which have a serious problem for the environment (Shahid *et al.,* 2017; Sonone *et al.,* 2020). The Cr reduction from Cr VI to Cr III is acting as an oxidizing agent in plant cells. It is a serious problem for plants as it damages growth and development due to phytotoxicity (Kumar *et al.,* 2019).

Various studies showed that the Cr toxicity affects the physiological mechanisms in wheat (González *et al.,* 2017), rice (Kabir, 2016) and ornamental plants (Liu *et al.,* 2017). The phytotoxicity of Cr firstly inhibits seed germination (Habiba *et al.,* 2019). Other morphological and physiological processes affected by Cr toxicity are roots damaging, nutrient uptake, photosynthesis, plant growth, flower quality and crop yield (Habiba *et al.,* 2019a). The plant produced reactive oxygen species (ROS) due to Cr toxicity which affect plant physiological processes (Farid *et al.,* 2017). The plant has its own antioxidative defense mechanism which is activated in response to ROS which improves the plant tolerance. The response of defense mechanism decreases in the higher Cr concentration due to the overproduction of ROS (Rizwan *et al.,* 2017; Rani *et al.,* 2020). So, the damaged physiological processes due to oxidative stress decreases plant growth. The different plant species have shown different morphological and physiological responses to Cr toxicity (Sallah-Ud-Din *et al.,* 2017). The toxic limit for plants is about 5 to 100 ppm in soil. Normally, the crops accumulate up to 1 ppm Cr without any physiological stress while the uptake depends on Cr present in soil (Shanker *et al.,* 2005; Wakeel *et al.,* 2020).

Marigold (*Tagetes patula* L.) is a famous annual ornamental plant belonging to the family Asteraceae present in many parts of the world (Aberer, 2008; Sonmez *et al.,* 2017). India and China are the most important marigold producer countries (Xia *et al.,* 2006). It is an important medicinal floriculture crop cultivated successfully throughout the year in varied soil

and climate conditions. It is commonly grown in pot and garden for aesthetic beauty. It is used in many social and religious functions in different forms (Abbas *et al.,* 2019). Marigold has great economic potential as a cut-flower. It is also used as a loose flower in different industrial sectors as a raw material. It is an important source of carotenoids and biologically active compounds (Netam, 2017). The flavonoids and saponins present in the marigold act as antispasmodic and antimicrobial commonly used by the pharmaceutical industry. The antioxidative activities and meat quality in broiler are enhanced by dietary supplements carrying marigold extract (Mehta *et al.,* 2012; Foroutankhah *et al.,* 2019). The natural food colorants present in the marigold also used in preparing milk derivatives, bread, vegetable oil, and pasta. The xanthophyll is used in poultry feed to intensify the yellow colorant of egg yolks and broiler flesh (Coelho *et al.,* 2017). The lutein present in petals used in the preparation of eye ailments and some other pigments are the important compounds of high-grade cosmetics and perfumes. The natural colorants of marigold also used as dying in the textile industry (Al-Saadi, 2009; Harlapur *et al.,* 2020). It is also used as a cover crop or companion crop to avoid the attack of nematodes because of its allelopathic effect. The allelochemical present in marigold extract used control of the attack of nematodes in crops (Tibugari *et al.,* 2012; Mokrini *et al.,* 2018).

Fulvic acid (FA) and humic acid (HA) are the major components of humic substances, they are straw-colored organic acids derived from natural organic matters (Ali *et al.,* 2015). It has highly soluble at low pH media and low molecular weight, so the agronomic efficiency of FA was higher as compared to HA (Ali *et al.,* 2018). FA containing more carbon-poor and oxygen-rich functional groups. It is also contained macro-nutrients, micronutrients, and amino acids, which perform all physiological activities (Wang *et al.,* 2019). The soil or foliar application of FA improves resistance in plants (Elrys *et al.,* 2020). They also intensify the plant biomass by increasing water use efficiency and decreased stomatal conductance (Yang *et al.,* 2017). The proline contents in wheat were increased with the foliar application of FA (Ali *et al.,* 2015). The FA reduced heavy metals mobility in soil (Li *et al.,* 2019). While the role of FA in heavy metal detoxification in ornamental plants was not extensively reported. The plant genotypes respond differently to heavy metal and humic substances due to genetic variability (Haider *et al.,* 2017). Very litter work has been reported on the role of FA on plant physiology in Cr stress. The aim of this study was to assess the physio-morphological responses of marigold genotypes to Cr toxicity under the foliar application of FA.

MATERIALS AND METHODS

The pot experiment was conducted in the wirehouse of Saline Agriculture Research Centre, University of Agriculture, Faisalabad. Before one month of sowing, a subsample of soil was used for the physicochemical analysis (Table 1), then the soil was spiked with 8 mg kg^{-1} Cr concentration by using potassium dichromate.

Table 1. **Properties of soil used for the pot experiment.**

Texture	**Clay loam**
Sand (%)	27.00
Silt (%)	21.00
Clay (%)	52.00
pH	6.73
ECe (dS m^{-1})	2.97
SAR $(mmol^{-1})^{1/2}$	6.80
Organic matter (%)	0.33
Available P (mg kg^{-1})	2.33
HCO_3 (mmol L^{-1})	3.79
Cl^- (mmol L^{-1})	2.97
$SO4^{2-}$ (mmol L^{-1})	9.67
Ca^{2+}+Mg^{2+} (mmol L^{-1})	3.30
Na^{2+} (mmol L^{-1})	3.50
K^+ (mmol L^{-1})	0.07

The two marigold (*Tagetes patula* L.) genotypes named *Striped marvel* and *Inca* were grown as a test crop. The seeds were sown in 2 kg soil filled in plastic pots. The full recommended dose of phosphate (P), potassium (K) and a half dose of nitrogen (N) were applied at sowing time and half after 40 d of the first application. The fulvic acid (FA) was applied as a foliar treatment 0, 2, 4, 6 and 8 mg L^{-1} with three replications.

***Plant harvesting*:** After three months of treatments, the shoots were harvested above 1 cm from the soil surface and roots were removed from the pots. The growth parameters including shoot length (SL) and root length (RL), fresh shoot weight (FRW) and fresh roots weight (FRW), dry shoots weight (DSW) and dry roots weight (DRW), number of flower $plant^{-1}$ (NoF), size of the flower (SoF) were recorded. The weight balance used for the fresh shoot and root weight then oven-dried at 65°C till constant weight for dry weight and the meter rod was used to measure the shoot and root length.

***Measurement of Photosynthetic Parameter*:** After three months of treatments, the chlorophyll and carotenoid contents measured from upper fully extended leaves by using a spectrophotometer after extracting the pigments in acetone. At 4 °C under darkness, the aqueous acetone (85% v/v) was used to continuous shake until the color had completely colorless and centrifuged at 4000 rpm for 10 min. The light absorbance 663, 644 and 452.5 mm was determined from the supernatant solution by spectrophotometer (Metzner *et al.,* 1965). The protocol defined by Lichtenthaler, (1987) was used to measure photosynthetic pigments and carotenoids.

***Determination of Reactive oxygen species*:** The electrolyte

leakage (EL) was measured with EC meter by the protocol defined by (Dionisio-Sese and Tobita, 1998). Firstly, The 5 mm length of leaves was dipped in a test tube containing distilled water. Then EC1 was recorded from the test tubes incubated in a water bath after 2 h at 32 ºC. For electrolyte leakage, plant samples were autoclaved for 20 min at 121 ºC for the destruction of plant tissues and EC2 was recorded. The following formula was used to calculating the EL.

$$\text{Electrolyte leakage} = \frac{(EC1)}{(EC2)} X100$$

The hydrogen peroxide (H_2O_2) was measured by the protocol defined by Jana and Choudhuri, (1981). The 3ml phosphate buffer, 50mM concentrated phosphate having pH 6.5 was used to homogenized the 0.25 mg leaves and roots then centrifuged for 25 min. Later, the H_2SO_4 (20% (v/v)) along with 1 mL titanium sulphate was centrifuged for 15 min with 3 mL supernatant solution. The light absorbance at 410 mm was determined. The hydrogen peroxide contents were calculated by the coefficient of extinction 0.28 μmol^{-1} cm^{-1}. While the 5 mL trichloroacetic acid (0.1%) was used to homogenize the 0.25 mg leaves and roots samples. In short, the reaction of thiobarbituric acid (TBA), which was modified by Zhang and Kirkham (1994) was used to measure the malondialdehyde (MDA) using the method defined by Heath and Packer (1968).

***Determination of antioxidant enzymes*:** The spectrophotometer was used to measure the antioxidant enzymes; superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) activity in marigold genotypes. The fully extended uppermost leaves and roots were taken to analyze the antioxidant enzymes. Firstly, the 5 mM phosphate buffer having 7.8 pH was used to homogenize the roots and shoots samples. Then the intensity of the supernatant solution was calculated at the required absorbance. The SOD and POD activities were measured at 560 nm and 470 nm by the method described by Zhang (1992), respectively. And the method described by Aebi (1984) was used to measure the CAT activity in marigold genotypes. Briefly, the spectrophotometer at 240 nm was used to measure the CAT activity of the mixture of enzyme extract carrying 0.05 M phosphate buffer (pH 7) and 10 mM H_2O_2. The APX activity was measured at a spectrophotometer at 290 nm from the mixture of 0.1 mL enzyme extract, 0.05 M phosphate buffer, 0.1 mM EDTA and 0.3 M H_2O_2 protocol defined by Nakano and Asada (1981).

***Determination of Cr concentration*:** The atomic absorption spectrophotometer (Analytik Jena, Germany) was used to measure the Cr concentration in marigold genotypes. Briefly, the 10 mL diacid solution ((NHO_3: $HCLO_4$) (3:1, v:v)) was used for the digestion of (1.0 g each) plant samples. After digestion, the Cr concentration was measured as followed by Ehsan *et al.,* (2015).

The phytoextraction coefficient (PEC) is the ratio of Cr in the plant over total Cr in soil, determined by the method described by Henry (2000). Using the translocation factor (TF%), the ability of plant for Cr translocation from root to aerial parts was measured. The relative biomass yield (RBY) and Cr bioaccumulation (BF) were calculated by the following formula followed by Coelho *et al.,* (2017)

$$\text{RBY} = \frac{\text{Dry biomass with FA}}{\text{Dry biomass without FA}} \text{ X } 100$$

$$\text{BF} = \frac{\text{Metal in the plant part (shoot or root)}}{\text{Metal in total plant}} \text{ X } 100$$

***Statistical analysis*:** The data were evaluated by using Statistix 8.1 statistical software package and the differences between the treatments were checked by using Tuckey's test at the 5 % probability level.

RESULTS

***Growth and productivity*:** The effect of fulvic acid on marigold genotypes was observed on growth parameters under Cr stress. The plant morphological attributes significantly increased with increasing fulvic acid concentration (Table 2). The result showed that more SL and RL, FSW, FRW, DSW, DRW, (NoF) and (SoF) were

Table 2. Agronomic attributes of marigold genotypes grown in Cr stress under different level of fulvic acid.

Fulvic acid (mg/L)	Genotypes							
	Striped marvel	Inca	Striped marvel	Inca	Striped marvel	Inca	Striped marvel	Inca
	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)		Number of flowers	
0	34.11f	33.98f	55.94g	53.88h	4.22f	4.11g	5.32d	5.17e
2	34.19f	34.08f	56.74f	54.16h	4.23f	4.14g	5.34d	5.19e
4	36.98d	36.57e	61.17d	60.19e	5.69d	5.45e	5.83b	5.70c
6	39.58b	39.12c	64.47b	63.31c	5.95ab	5.82c	5.99a	5.88b
8	41.29a	41.09a	65.64a	64.50b	5.99a	5.89bc	6.01a	5.99a
	Root length (cm)		Root Fresh weight (g)		Root dry weight (g)		Size of flower (cm)	
0	8.15e	8.08e	4.22f	4.11g	1.16f	1.10f	5.18f	5.11f
2	8.16e	8.11e	4.23f	4.14g	1.17f	1.11f	5.20f	5.12f
4	9.62c	9.31d	5.69d	5.45e	1.95d	1.86e	5.84d	5.69e
6	9.94ab	9.84b	5.95ab	5.82c	2.15ab	2.02c	6.14b	6.03c
8	10.03a	9.92ab	5.99a	5.89bc	2.21a	2.10b	6.29a	6.18ab

observed in marigold genotype striped marvel in comparison with the Inca. The morphological traits were significantly increased at FA 8 mg L^{-1} as compared to FA 0 mg L^{-1} in both genotypes. The FA application enhanced shoot length 21 % and root length 23 % as compared to control. The SDW and RDW were increased up to 46 % and 89 % respectively at FA 8 m L^{-1} in striped marvel and increased 41 % and 91 % respectively, at FA 8 mg L^{-1} in Inca.

***Photosynthetic pigments assay*:** The Cr stress decreased photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids) in marigold genotypes (Fig. 1). The striped marvel produced more photosynthetic pigments as compare to Inca. The application of FA 8 mg L^{-1} significantly improved the chlorophyll pigments in both genotypes as compared to FA 0 mg L^{-1}. The total chlorophyll contents and carotenoids were enhanced up to 79 % and 86 %, respectively, at FA 8 mg L^{-1} as compared to FA 0 mg L^{-1} in striped marvel. While, 82 % and 89 % respectively, at FA 8 mg L^{-1} in Inca.

***Reactive oxygen species*:** The concentration of reactive oxygen species EL, H_2O_2, and MDA contents were measured marigold genotypes by applying FA (Fig. 2). The results showed that genotypes produced maximum EL, H_2O_2 and MDA contents at FA 0 mg L^{-1} and the contents were significantly decreased with the foliar application of FA 8 mg L^{-1}. It is also noticed that the maximum EL, H_2O_2 and MDA contents were produced in Inca as compared to striped marvel. The EL content in leaves was decreased 30 % in striped marvel while 38 % in Inca as compare to FA 0 mg L^{-1}. The H_2O_2 and MDA contents in leaves were decreased by 55% and 74% respectively at FA 8 mg L^{-1} in striped marvel, while 52% and 68 % respectively at FA 8 mg L^{-1} in Inca.

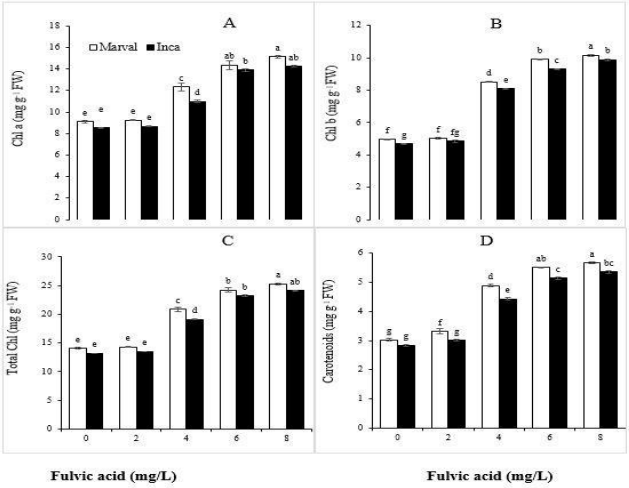

Figure 1. Response of marigold genotypes Marvel and Inca to Cr stress under different levels of Fulvic acid on chlorophyll a (A), chlorophyll b (B), total chlorophylls (C) and carotenoids (D). Values are demonstrated as means of three replicates along with standard error. Different small latter shows significant difference at P < 0.05.

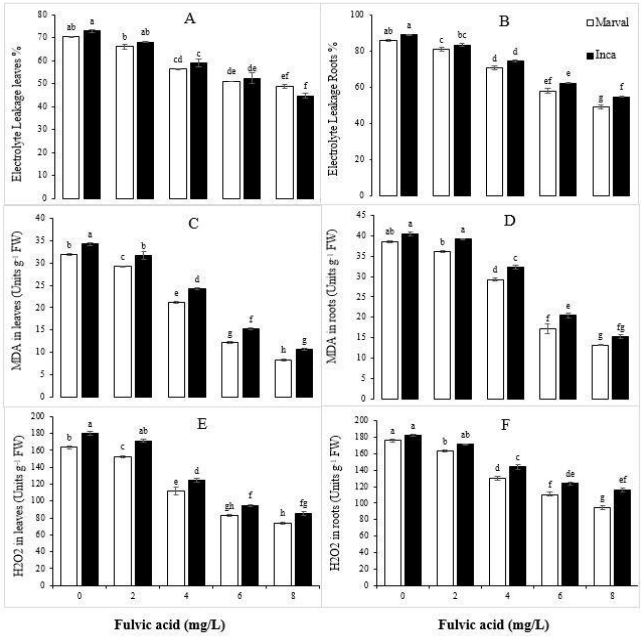

Figure 2. Response of marigold genotypes Marvel and Inca to Cr stress under different levels fulvic acid on Electrolyte leakage in leaves (A), Electrolyte leakage in leaves (B), H_2O_2 in leaves (C), H_2O_2 in roots (D), MDA in leaves (E) and MDA in roots (F). Values are demonstrated as means of three replicates along with standard error. Different small latter shows significant difference at P < 0.05.

***Antioxidative Enzymes Activities*:** The effect of FA was assessed on the activities of antioxidative enzymes (SOD, POD, CAT, APX) in marigold genotypes. The Cr stress was significantly disturbed the activities of these enzymes. The activities of antioxidative enzymes in marigold genotypes significantly improved with the foliar application of FA (Fig. 3). The maximum activities were observed in Marvel as compared to Inca. The FA 8 mg L^{-1} significantly improved the SOD, POD and CAT activities as compared to FA 0 mg L^{-1}. The APX activities in leaves and roots were increased by 81 % and 60 % respectively, at FA 8 mg L^{-1} in striped marvel and increased by 84 % and 66 % respectively, at FA 8 mg L^{-1} in Inca.

Cr Concentration in Plant: The results showed that the Cr concentration in roots, shoots, and flower of marigold genotypes were significantly ($P< 0.05$) reduced with the foliar application of FA (Table 3). Striped marvel accumulated less Cr as compared to Inca. The maximum Cr concentration 6.35 mg kg^{-1} was uptaken by Inca at FA 0 mg L^{-1}, while decreased to 5.71 mg kg^{-1} at the FA 8 mg L^{-1}. The phytoextraction, translocation, and bioaccumulation of Cr in plant parts were also decreased at FA 8 mg L^{-1} in comparison to FA 0 mg L^{-1} in both genotypes.

Table 3. Chromium in roots, shoots, flower, total Cr, Phytoextraction coefficient (PEC), Bioaccumulation (BA) and Translocation factor (TF) of marigold genotypes grown in Cr stress under different level of fulvic acid.

Fulvic acid (mg/L)	Genotypes									
	Inca	Striped marvel	Inca	Striped marvel	Inca	Striped marvel	Inca	Striped marvel	Inca	Striped marvel
	Cr in Roots		Cr in Shoots		Cr in flower		Total Cr		PEC	
0	3.383a	3.27b	2.63a	2.55b	0.34abc	0.38a	6.35a	6.21bc	0.79a	0.77bc
2	3.380a	3.26b	2.62a	2.54b	0.32abc	0.35ab	6.33ab	6.16cd	0.79ab	0.77cd
4	3.27b	3.18c	2.51b	2.43c	0.28cd	0.31bc	6.07d	5.93e	0.75d	0.74e
6	3.20bc	3.10de	2.44c	2.36de	0.20ef	0.24de	5.85e	5.71f	0.73e	0.71f
8	3.15cd	3.05e	2.39cd	2.31e	0.16f	0.20ef	5.71f	5.57g	0.71f	0.69g
	Translocation factor (shoot to root)		Translocation factor (flower to shoot)		Bioaccumulation in Roots		Bioaccumulation in Shoots		Bioaccumulation in flower	
0	0.77a	0.78a	0.13ab	0.15a	56.26a	56.14a	43.73a	43.85a	5.40ab	6.21a
2	0.77a	0.77a	0.12abc	0.13ab	56.30a	56.19a	43.69a	43.80a	5.16abc	5.74a
4	0.76a	0.76a	0.11bcd	0.12ab	56.53a	56.66a	43.46a	43.33a	4.66bcd	5.29ab
6	0.76a	0.76a	0.08ef	0.10cde	56.75a	56.73a	43.24a	43.26a	3.55ef	4.20cde
8	0.76a	0.75a	0.06f	0.08def	56.79a	56.89a	43.20a	43.10a	2.92f	3.71def

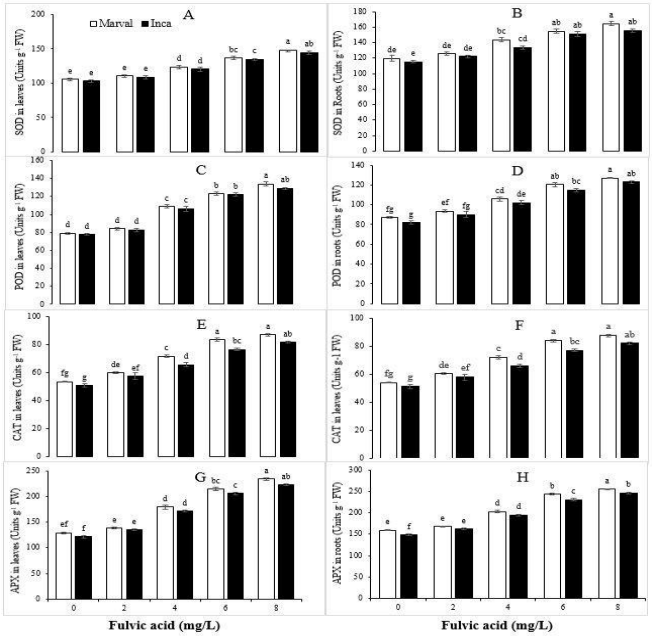

Figure 3. Response of marigold genotypes marvel and Inca to Cr stress under different levels of fulvic acid on SOD in leaves (A), SOD in roots (B), POD in leaves (C), POD in roots (D), APX in leaves (E), APX in roots (F), CAT in leaves (G) and CAT in roots (H). Values are demonstrated as means of three replicates along with standard error. Different small latter shows significant difference at P < 0.05.

DISCUSSION

In this experiment, we assessed the effect of FA in the alleviation of Cr toxicity in marigold genotypes. The foliar application of FA significantly enhanced the morphological parameter in both genotypes (Table 2). The toxic effects of Cr were significantly reduced with the foliar application of FA in both marigold genotypes in a dose-additive manner. And the nutrient available to plants were also increased. Many reports are published on the role of FA in plant physiology (Yang *et al.,* 2017; Li *et al.,* 2019). The availability of essential nutrients to plants decreased due to competition with Cr. This might be the reason for the less biomass production under Cr stress (Elrys *et al.,* 2020). The RBY was also increased in both genotypes with increasing FA concentration (Table 4).

Table 4. Relative biomass yield (RDY, %) of marigold genotypes grown in Cr stress under different level of fulvic acid

Treatments	Fulvic acid (mg/L)				
	0	**2**	**4**	**6**	**8**
Striped marvel	100	103.10	109.54	133.71	133.80
Inca	100	103.43	110.90	131.71	132.00

The reduced plant biomass might be due to reduced plant and soil enzymatic activities in Cr stress and the delayed cell division, cell elongation or completion in the cell cycle might be another reason for less biomass production (González *et al.,* 2017). The higher morphological growth due to more photosynthesis and active antioxidative defense mechanisms might be the reason of increased RBY (Coelho *et al.,* 2017). The FA application also improves the photosynthetic pigments and carotenoids in both genotypes (Fig. 1). The Cr toxicity in plants was significantly reduced the chlorophyll contents that play important role in photosynthesis (Farid *et al.,* 2017). The possible reason might be the inhibition of chlorophyll biosynthesis due to inhibition of enzymes because

of the Cr toxicity (Anjum *et al.,* 2016). The disturbance in biosynthesis pathways due to reduced activities of biosynthetic enzymes might be the reason for fewer chlorophyll contents. The less photosynthetic pigments might be due to the chloroplast destruction by overproduction of ROS (Habiba *et al.,* 2019). The effective role of FA on chlorophyll was already reported in maize (Anjum *et al.,* 2011). The decreased uptake of Cr in the plant due to FA might be the reason for increased chlorophyll contents and carotenoids in both genotypes. The reduction in chloroplast damage and ROS production and improved water transport efficiency due to the application of FA might be another reason for increased chlorophyll content in the plant (Ali *et al.,* 2018).

The production of reactive oxygen species (ROS) was increased in both genotypes due to Cr toxicity (Fig. 2). Different reports show that the antioxidant defense mechanisms and other stress tolerance mechanisms in plants repair the damage up to a certain limit of toxicity (Abbas *et al.,* 2019). The overproduction of ROS due to high Cr toxicity slow the antioxidant defense mechanisms in wheat (Arshad *et al.,* 2016). The Cr toxicity damage to cell wall due to the overproduction of ROS and decreased to antioxidative enzyme activities in a plant (Gonzalez *et al.,* 2017). Previous reports show that the overproduction of ROS alters the plant cell shape (Ali *et al.,* 2018). The counter-response of plant defense mechanisms under high Cr toxicity is very low due to overproduction of ROS which led to damage of plant physiological processes (Abtahi *et al.,* 2017). The EL, MDA and H_2O_2 contents significantly decreased with the foliar application of FA by improving the antioxidant defense mechanisms in both marigold genotypes, which might be due to scavenging or reduced ROS production in the plant. The cellular lesions in the plant have increased, possibly due to the reduced cellular integrity affected by ROS (Ali *et al.,* 2013). The foliar application of FA improved antioxidative defense mechanisms due to improved activities of antioxidative enzymes which reduced ROS and membrane ultradestruction in plants might be due to adsorption of the free redials (Yang *et al.,* 2017).

The antioxidant enzyme activities decreased in both marigold genotypes due to Cr toxicity. While the FA application significantly increased these antioxidant enzyme activities (Fig. 3). They improved cellular structure, integrity, and stability by decreasing the ROS activities in the plant (Shahid *et al.,* 2017). Similar results were reported by Rizwan *et al.,* (2017). The FA activates antioxidative defense mechanisms that improve metal detoxification in plants. The adsorption of ROS and the reduced uptake of Cr in plant parts might be the reason for increased antioxidant enzyme activities in the plant. The FA improves the synthesis of the enzyme which significantly improves the activities of SOD, POD, and CAT (Anjum *et al.,* 2011). The increasing concentration of organic acids significantly increased the nutrients concentration in plant parts might be the reason for more antioxidant enzymes activities in a plant (Sonmez *et al.,* 2017).

The Cr concentration in roots, shoot and flower translocation and phytoextraction were significantly decreased with the application of FA in both marigold genotypes in a dose-additive manner (Table 2). Many reports are in line with our findings (Sallah-Ud-Din *et al.,* 2017; Wang *et al.,* 2019). The heavy metal uptake and translocation are varied from species to species due to the genetic variability of the crop (Tan, 2000). The genetic response of plants toward abiotic stress was varied from genotype to genotype (Sonmez *et al.,* 2017; Habiba *et al.,* 2019a). Cr accumulation by plants in their different parts is greatly varied due to plant defense mechanisms (Van der Ent *et al.,* 2013). The plant roots produce exudates which can be helpful for the uptake of nutrients by changing the redox condition or by changing the pH, so nutrients become soluble and then the roots took up the elements might be the reason for less Cr accumulation (Custos *et al.,* 2020). The rhizosphere is acidic due to secreted protons of plants' roots, thus metal dissolution is increased (Gerke, 2015). The extracellular exudates can change the pH or redox conditions which can affect the metal available to the plant might be the reason for low Cr uptake (Ma *et al.,* 2016). The ameliorative role of FA towards Cr might be the reason for low Cr uptake in plants (Ali *et al.,* 2018). The less Cr translocation was observed in both genotypes with the application of FA. The reason might be due to the formation of compartmentation in root cells or insoluble Cr salts in roots. The Cr entry into cytoplasm might be prohibited by the FA- induced changing in the cell membranes (Ali *et al.,* 2015). The FA simulate the plant for the production of root exudates for the binding of these heavy metals might be another reason for low Cr uptake by increasing FA application (Li *et al.,* 2019). The results show that striped marvel was more sensitive toward Cr as compare to Inca because they uptake Cr concentration in their roots, shoot and flowers.

***Conclusion*:** The results of this present study showed the importance of FA under Cr stress. In Cr toxicity, the plant morphological parameter, photosynthetic pigments, and antioxidative enzyme activities were significantly decreased and reactive oxygen species was increased. While the application of FA was significantly increased the plant morphological parameter, photosynthetic pigments and antioxidative enzyme activities increased and decreased the reactive oxygen species in a dose-additive manner. The Cr translocation from root to shoot, shoot to flower were also decreased in both marigold genotypes (striped marvel and Inca). The results of the study show that the foliar application FA is beneficial to alleviate Cr toxicity in marigold. However, detailed studies are needed to understand the role of FA to alleviate heavy metal stress.

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