

## ALLEVIATION OF HEAVY METAL TOXICITY (ARSENIC AND CHROMIUM) FROM MORPHOLOGICAL, BIOCHEMICAL AND ANTIOXIDANT ENZYME ASSAY OF WHEAT (*TRITICUM AESTIVUM* L.) USING ZINC-OXIDE NANOPARTICLES (ZNO-NPS)

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### Abstract

In Pakistan, wheat is a major staple crop that is also considered as energy crop. At present, it has been developed as animal fodder, malt goods and human consumption. Under the heavy metal stress, the growth and net production of wheat is compromised. Arsenic and Chromium are known as the utmost injurious heavy metal to different cereal crops that affect their physiological and yield attributes. In roots, high quantities of heavy metals are particularly necessary for fodder, herbal crops and cereals. In most recent years, Zinc oxide nanoparticles are known as active entities that are commercially used to enhance the growth, development and production of wheat crop to get economic benefits under stresses. However, in Pakistan, Zinc oxide nanoparticles interaction in Wheat. For this purpose, pot experiments were performed to evaluate the response of Zinc oxide nano particles under heavy metals (chromium and arsenic) stress on two wheat varieties (Aas 2011 and Faisalabad 2008) during 2018-20. Heavy metals (Cr and As 10ppm) were added to plants as rooting medium before sowing. After 14 days of germination, 10, 20, and 30 ppm of ZnO nanoparticles were applied one time as foliar spray. Four replications were used in these experiments using completely randomized design (CRD). Results indicated that the applications of ZnO-NPs, reduced the effect heavy metals including As and Cr concentration in relation to various morphological, physio-chemical, antioxidant enzyme activities and yield parameters in two varieties of wheat. Compared to Aas (2011), variety Faisalabad (2008) showed better performance for these attributes. Results also narrated that heavy metal (As) decreased various attributes of morphology, physiology and yield of both wheat varieties. These findings indicated that the use of ZnO nanoparticles can improve the biochemical, morphological, antioxidant and yield of wheat varieties under heavy metal stress. Applications of ZnO-NPs also showed significance in lowering the toxicity of heavy metals from.

**Key words:** Wheat, Nanoparticles, Growth, Physiology, Yield, Arsenic and Chromium.

### Introduction

Cereals have a significant part in human nutrition, whether they are used in cooking or as a source of flour for baking. Botanically, they are a member of the grass family (Gramineae), which also includes rye, oats, barley, millets, sorghum, and wheat (Mir *et al.*, 2019). In Pakistan wheat is the fundamental food of the province of Punjab that provide more than 70% of all the native production. In the Punjab region of Pakistan wheat is the principal diet, which provides higher than 75% of the whole domestic crop yield. To generating foodstuffs for the complete country it requires 43.7% work power. During the winter period of 2013–2014 wheat was cultivated on land of about 9 million hectares and complete output 25.3 million tons (Ali *et al.*, 2017).

Wheat is imperative cereal crop also major source of nutrients and is widely consumed and grown in Pakistan. It contains almost 1.5-2% fat, 60-80% protein, 2-3% mineral matter and 2-2.5% glucose but these may differ or vary from area to area as well as variety to variety (Munns, 2008). Wheat is a winter crop that is grown throughout Pakistan. In Pakistan, wheat production is around 23888 kg ha<sup>-1</sup> that is very low in wheat growing regions in all over world (Turki *et al.*, 2012). The reason behind the less yield of wheat include less and inequity use of fertilizer, climatic condition of area, less fertility status of soil, deficiency of micronutrient and macronutrient and illiteracy and utilization of latest technology by the former (Kalhor *et al.*, 2016). Wheat (*Triticum aestivum*) is used in several other products for example straw particle (wood) used in

paper, hair conditioner, kitchen cabinets, ink, charcoal, adhesive on postage stamps medical swab, milk replacer, decomposable golf tees and Decomposable plastic intake utensils etc. (Pathak & Shrivastav, 2015).

Heavy metal contaminants including lead, cadmium and mercury are challenging to remove from sand and water because they are difficult to break down chemically into harmless constituents (Venkatachalam *et al.*, 2017). As it has a direct influence on ecosystem and health of humans, Pb and Cd metal ion contamination of agriculture and water are one of the major global issues (Srivastava *et al.*, 2017). The crop of wheat regained high quantity of dense metals from drenching with contaminated water and generate various threats to wellness of human beings (Kishor *et al.*, 2020). Many dense metals across food web transmitted to different medium due to their aggregation in agriculture products. In the crop–soil line the bio concentration factor (BCF) of numerous dense metals has been recognized, principally in basic worldwide fundamental crops for example corn and wheat (Bezie *et al.*, 2021).

Bioscience and biomedicine utilise nanoparticles (NPs), which are ultrafine particles with sizes between 1 and 100 nm (Salata, 2004). NPs interaction with plant and environment is not much explored. Thul *et al.*, (2013) reported both positive as well as harmful effects of through application of NPs in many crops. ZnO nanoparticles have recently been shown to be a possible adsorbent for the effective removal of heavy metals from aqueous solution by Azizi *et al.*, (2017) and Kumar *et al.*, (2021). When compared to bulksized particles of the same minerals, NPs

have been found to be particularly effective at removing metallic ion pollutants from soil solutions and waste waters because of their large surface area and surface area-to-volume ratio (Habuda-Stanic & Nujic, 2015). Zinc oxide nanoparticles play a significant role to promote growth and yield of different crops (Rizwan *et al.*, 2019). There are few components which influence zinc oxide toxicity and zinc availability in soil like soil type, soil pH and the plant species (Garcia-Gomez, 2018).

## Material and Methods

Pot experiments were conducted at the Botanical Garden of University of Gujrat, Gujrat, Pakistan. Two wheat varieties were used in this experiment i.e. Faisalabad (2008) and Aas (2011). Plants were given arsenic as well as chromium in the form of arsenic and chromium oxide respectively. Treatment levels were 0 (control) and 10 ppm by plant rooting media. ZnO nanoparticles were applied (foliar spray) after some days of HMs stress. Early in the morning, a hand sprayer was used to apply ZnO nanoparticles. Four replicates were used in each experiment using CRD (Completely Randomized Design). Data was collected at the vegetative and maturity stages.

$$\text{Area of leaf (cm}^2\text{)} = \frac{\text{Leaf length maximum} \times \text{Leaf width maximum} \times 0.75}{(\text{Correction factor} = 0.75)}$$

Biochemical parameters including Chlorophyll *a*, *b*, as well as carotenoid concentration are deliberated by Arnon procedure as designated by Nazeer *et al.*, (2020). In Krishnaveni techniques which were elaborated by Nazeer *et al.*, (2020) in quality attributes such as carbohydrates were determined. Total soluble proteins were calculated by Lowery *et al.*, (1951) method. Antioxidant activities such as Superoxide dismutase (SOD) was detected through estimating restriction in nitroblue tetrazolium photo reduction according to Giannopolitis and Ries method as used by Ullah *et al.*, (2015). Catalase (CAT) was determined by Aebi method as used by Aziz *et al.*, (2014). Peroxidase (POD) activity was recorded as described by Hussain *et al.*, (2020) using the Chance and Maehly procedure. By using the free radical scavenger and lipid peroxidation analysis proline contents of leaf was calculated. Bates *et al.*, (1973) method was used to measure the proline contents of leaf. Total phenolic contents (TPC) were measured with the help of Folin Ciocalteu's method (Yim *et al.*, 2010) using Gallic acid as the standard. For the examination of Glycine betaine, completely developed uppermost leaves from plants growing under normal and water-stressed circumstances were taken, and Grieve & Grattan (1983) procedure was followed.

To calculate the yield, seeds of each plant were weighed by electronic balance manually. Afterwards average value was determined. Spikes numbers on each plant was analyzed and calculate their mean value after that utilized it for further work.

**Green synthesis of ZnO nanoparticles:** In University of Gujrat in Pakistan, fresh *Aloe vera* leaves were gathered.

**Treatments:** There were following levels of treatments:

- T0 Control 0 ppm (As, Cr and ZnO-NPs)
- T1, 10 ppm As
- T2, 10 ppm Cr
- T3, 10 ppm ZnO-NPs
- T4, 20 ppm ZnO-NPs
- T5, 30 ppm ZnO-NPs
- T6, 10 ppm ZnO-NPs + 10 ppm As
- T7, 20 ppm ZnO-NPs + 10 ppm As
- T8, 30 ppm ZnO-NPs + 10 ppm As
- T9, 10 ppm ZnO-NPs + 10 ppm Cr
- T10, 20 ppm ZnO-NPs + 10 ppm Cr
- T11, 30 ppm ZnO-NPs + 10 ppm Cr

**Analytical tests:** Morphological parameters such as lengths of shoot and root were dignified with the help of iron meter scale. Root and shoot fresh and dry weights were noted with electric balance. Each plant's leaves were counted, and the mean value for each plant was then determined. Using the Carelton & Foote (1965) formula, the area of the leaf plant was calculated. The formula used is:

Small leaf bits were cut. Using electrical balancing, 35g of cut components were weighed. These leaves were boiled in 100ml of pure water. Purification and storage of the recovered substance followed. 30 ml of Extract of *Aloe vera* was utilised as a reducing agent to convert zinc nitrate into ZnO-NPs. This is why 3g of zinc-nitrate was added to *Aloe vera* extract. The sample was then placed at 60°C while being stirred magnetically until it dried. The powder has been dried in a furnace at 400 degrees to eradicate any moisture (Manohar *et al.*, 2021). The structural and morphological characteristics of the sample were categorized using XRD, SEM and FTIR spectroscopy techniques.

## Statistical analysis

Using Ministat-C software, data were exposed to ANOVA for 27 variables along with 2 factors, namely years, locations, and means were associated through the Tukey's HSD test at P value of 0.05, having confidence interval of 95% (Silverman, 2018). Pearson's correlation coefficients were calculated from various attributes using MS-Excel (Version-2013).

## Results and Discussion

**Morphological characters:** Data pertaining to morphological parameters of two wheat varieties have been presented in Tables 1 & 2. Means squares from ANOVA specified that ZnO nanoparticles effect on heavy metal stressed plants on shoot and root length presented significant results ( $p < 0.05$ ) in (Table 1). However, the variation among Variety  $\times$  Years showed

non-significant ( $p>0.05$ ) outcomes. The highest length of shoot (cm) was observed in Faisalabad (2008) at T5 (ZnO 30 ppm) and low length (cm) was noted at T1 in Aas (2011) in 2019 as compare to 2020. Effect of heavy metal (10 ppm Cr) in combination with 10, 20, 30 ppm ZnO nanoparticles gave the best results of shoot length as compare to control (Fig. 1A). Maximum root length (cm) was recorded at T1(As 10ppm) in Aas (2011) in 2019 and minimum was at ZnO 10 ppm + As 10 ppm in Aas (2011) in 2020 (Fig. 1B). Ahmad and Gupta (2013), Pandey *et al.*, (2016) and Praveen *et al.*, (2018) have previously demonstrated the harmful effect of As on shoot and root lengths of mustard and rice crops, which might be connected to heavy metal-mediated disturbances in balance of water or mobilization of nutrient, one of the main factors in the decline in plant development caused by heavy metal stress is that it causes water balance to be disturbed, which creates a water deficit. There were also highly significant results ( $p<0.05$ ) of fresh and dry weight of shoot and root on the effect of as and Cr and ZnO nanoparticles treated plants at both years. On the other hand, the interaction between Variety  $\times$  Year, Treatment  $\times$  Year, Variety  $\times$  Treatment  $\times$  Year gave non- significant ( $p>0.05$ ) results (Table 1). It is remarkable to note that foliar application of ZnO NPs alone as well as with heavy metals, the weight of fresh shoot (g) decreases as compare to control at both years. However, maximum dry weight of shoot (g) was obtained at the ZnO nanoparticles addition along with different concentration of Chromium as compare to control at both years (Fig. 1C & D). Results from Fig. 2

A & B narrated that application of ZnO nano particles alone gave highest weight (g) of fresh root at both years as compare to control while maximum dry weight of root was recorded at ZnO 20 ppm + Cr 10 ppm in Aas (2011) in 2019. Arsenic toxicity has an effect on soybean plant growth in terms of shoot length as well as fresh and dry shoots and roots weights (Ahmad *et al.*, 2020). These findings support Jung *et al.*, (2017) findings in As-stressed rice seedlings. Data from Means square study presented the influence of heavy metals as well as ZnO nanoparticle on number of leaves also leaf area of two varieties of wheat were highly significant ( $p<0.05$ ) with its interactions. However, varietal response to leaf area showed non-significant ( $p>0.05$ ) outcomes (Table 2). Foliar application of ZnO (10, 20, 30 ppm) nanoparticle alone on two varieties of wheat gave best finding on leaf area and number of leaves at Faisalabad (2008) on both 2019 and 2020 years. Minimum results of number of leaves and leaf area were obtained on those plants in which heavy metals were applied individually and combination with ZnO Nanoparticles. Heavy metal Arsenic gave poor results on all morphological parameters of two varieties of wheat either singly or combination with ZnO nanoparticles effected wheat varieties. Abedin, *et al.*, (2002) found that as toxicity inhibited rice development, which is similar to our findings. Arsenic is thought to interfere with metabolism by reacting with the thiol groups of enzymes. Cell cycle capture and DNA synthesis inhibition and repair pathways have been proposed as causes of arsenic-mediated growth suppression (Ghani *et al.*, 2014).

**Table 1. Means squares (MS) from ANOVA for morphological characteristics of two varieties of wheat (*Triticum aestivum* L.) when exposed to foliar treatment of ZnO nanoparticles under stress of arsenic and chromium.**

Source	df	MS of shoot length (cm)	MS of root length (cm)	MS of shoot fresh weight (g)	MS of shoot dry weight (g)	MS of root fresh weight (g)	MS of root dry weight (g)
Variety	1	2.205 *	0.002***	0.869***	0.0004ns	0.0001***	0.0003**
Treatment	11	34.355 ***	0.001***	7.349***	0.001**	0.0003***	0.0002***
Year	1	84.614 ***	0.000 *	0.850***	0.0120***	0.0001*	0.0005**
Variety $\times$ Treatment	11	2.4961 ***	0.001***	1.187***	0.0003ns	0.0001***	0.0002***
Variety $\times$ Year	1	0.0507 ns	0.000 ns	3.569***	0.0005ns	0.0000ns	0.0001*
Treatment $\times$ Year	11	4.6400 ***	0.0002*	1.754***	0.0009**	0.0000ns	0.00005ns
Variety $\times$ Treatment $\times$ Year	11	2.7709 ***	0.0001ns	1.186***	0.0004ns	0.00003ns	0.00006ns
Error	144	0.6366	0.0001	0.03482	0.0003	0.000	0.00004
Total	191	665.430	0.065608	136.559	0.089728	0.011203	0.013084

ns=Non-significant; \*,\*\*,\*\*\*, Significant at 0.05, 0.01 and 0.001, respectively

**Table 2. Means squares (MS) from the analysis of variance (ANOVA) for morphological and physiological attributes of two different varieties of wheat (*Triticum aestivum* L.) when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress.**

Source	df	MS of leaf area/plant (cm <sup>2</sup> )	MS of number of leaves	MS of Chl a (mg/g)	MS of Chl b (mg/g)	MS of carotenoid content (mg/g)
Variety	1	72.459***	0.6302ns	0.00002***	0.0001***	0.25517***
Treatment	11	45.424***	12.789***	0.00008***	0.0003***	0.22774***
Year	1	147.420***	30.880***	0.00003 ***	0.0000***	1.44500***
Variety $\times$ Treatment	11	105.239 ***	4.6529***	0.00003***	0.00005***	0.03334***
Variety $\times$ Year	1	34.315 **	3.7969*	0.00001***	0.0000ns	0.04872*
Treatment $\times$ Year	11	9.901***	2.6529***	0.00005***	0.0000ns	0.06430***
Variety $\times$ Treatment $\times$ Year	11	7.630**	1.9332**	0.00005***	0.0000ns	0.02249**
Error	144	2.863	0.7899	0.000001	0.000003	0.00798
Total	191	2516.66	391.370	0.002722	0.004807	6.72497

Ns = Non-significant; \*,\*\*,\*\*\*, Significant at 0.05, 0.01 and 0.001, respectively

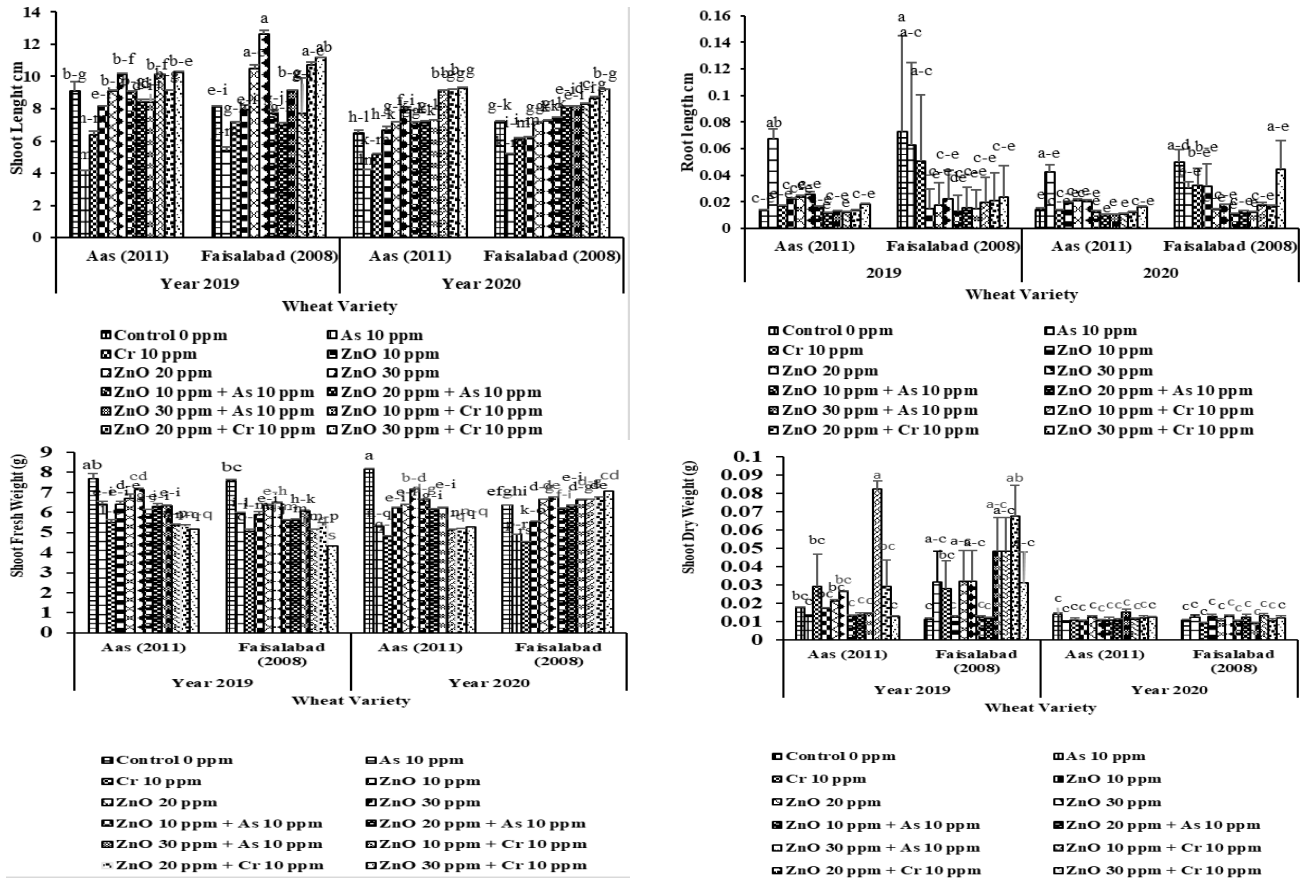


Fig. 1. Morphological attributes of two different wheat varieties (*Triticum aestivum* L.) when subjected to foliar treatment of ZnO nanoparticles under stress of arsenic and chromium. (A) Shoot length, (B) Length of root, (C) Fresh weight of shoot, (D) Dry weight of shoot.

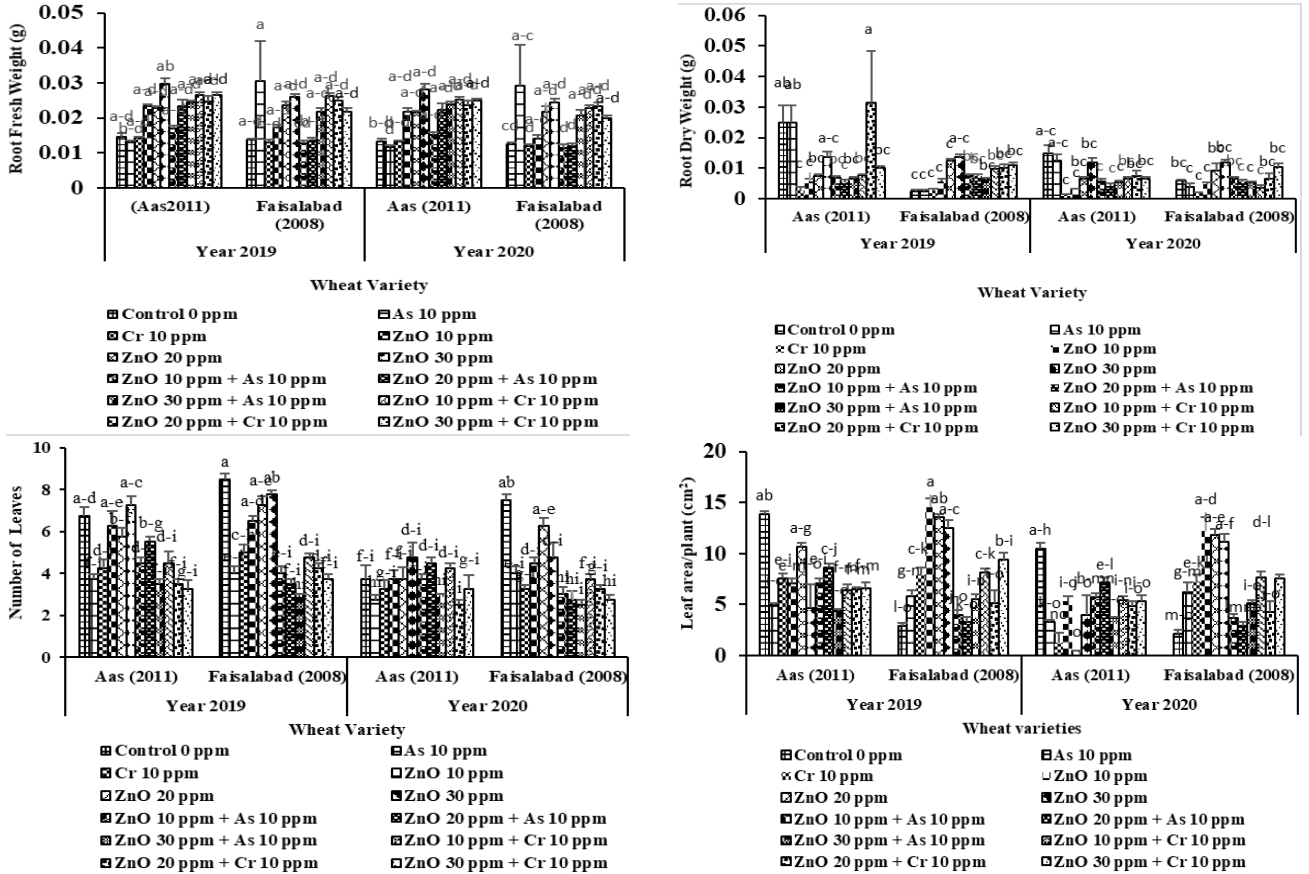


Fig. 2. Morphological attributes of two wheat varieties *Triticum aestivum* L. when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress. (A) Root fresh weight (g), (B) Root dry weight (g), (C) Number of leaves, (D) Leaf area/plant (cm<sup>2</sup>).

**Physiological characters:** Data from ANOVA for the influence of heavy metals combination with ZnO NPs on photosynthesis pigments including chlorophyll a, b and carotenoids have been illustrated in Table 2. Means squares analysis exposed that impact of heavy metals and ZnO NPs on photosynthetic pigments were highly significant ( $p < 0.05$ ) on wheat at both years. The interaction between varieties, year and treatments also gave highly significant results. Maximum contents of chlorophyll a and b (mg/g) were observed in Faisalabad (2008) at T11 (ZnO 30 ppm + Cr 10 ppm) and minimum contents (mg/g) were present in Aas (2011) at T1 (10 ppm As) at both years (Fig. 3A & B). However, highest contents of carotenoids were noted at Faisalabad (2008) at T11 (ZnO 30 ppm + Cr 10 ppm) in 2019 and T10 (ZnO 20 ppm + Cr 10 ppm) in 2020 and lowest content were recorded in T2 (20ppm As) at both years (Fig. 3C). Under As stress, soybean decreased the photosynthetic attributes in the plants. Arsenic has a decreased bioavailability because it affects nitrogen metabolism. Arsenic stress decreases nitrogen's bioavailability because it is a crucial component of chlorophyll, reducing a plant's chlorophyll concentration. Arsenic could be one of the primary causes of plants' reduced photosynthetic rate when it is present (Rahman *et al.*, 2007). Our findings of reduced pigments of photosynthesis and their rate are in agreement with earlier reports on a range of various arsenic-stressed plants (Namjoyan *et al.*, 2020). Our findings support Ahmad *et al.*, (2020), findings that treatment of ZnO-NP boosted rice plants' photosynthetic rates. This may be connected to metal nanoparticles' capacity to raise the synthesis of pigments, plant quantum yield, and the formation of chemical energy in the system of photosynthesis (Li *et al.*, 2020). Means square from ANOVA specified that heavy metals influences and ZnO-NPs on carbohydrates content gave significant results on all varieties of wheat. However, its interaction presented non-significant results (Table 3). Graphical representations showed that highest contents of carbohydrates were noted at foliar spray of ZnO nanoparticles (20ppm) at Aas (2011) at 2020 year. Overall results also narrated that application of ZnO nanoparticles gave best outcomes as compare to other at both years (Fig. 3D).

**Antioxidant activities:** Antioxidant enzyme activities on wheat varieties were present in (Table 3). Means square demonstrated the results of heavy metals and ZnO NPs impact on antioxidant enzymes activities such as catalase, peroxidase and superoxide dismutase were highly significant ( $p < 0.05$ ) on all varieties of wheat at both years with its interaction. While response of years on peroxidase activities showed non-significant ( $p > 0.05$ ) outcomes. Significant contents of catalase activities (Units/mg protein) were recorded at Cr (10ppm) in combination with ZnO nanoparticles (10, 20, 30ppm) and lowest contents were noted in control in 2019 at both varieties (Fig. 4A). Maximum peroxidase contents (Units/mg protein) were present in ZnO application with heavy metal (Cr)

combination at Faisalabad (2008) in 2019 year while minimum content was noted at control at both varieties (Fig. 4B). Highest contents of superoxide dismutase (Units/mg protein) were noted at ZnO nanoparticles (30 ppm) application signally and combination with heavy metal (10 ppm Cr) and low contents were observed at heavy metals application on both years at both varieties (Fig. 4C). Increased antioxidant enzyme activity against oxidative stress has also been connected to over-expression of the SOD, POD, and CAT isozymes (Talukdar, 2013). Similar results were reported when soybean plants (As-stressed) were supplemented with ZnO-NPs, which boosted defensive enzymes activity. The increased SOD, CAT, and POD activities caused by ZnO-NP in As-stressed soybean plants in accordance to findings of (Venkatachalam *et al.*, 2017) in *Leucaena leucocephala* seedlings under heavy metal stress. Many authors have found increased SOD, CAT and POD activities produced by ZnO-NP in many plants, including Tripathi *et al.*, (2015) in *Pisum sativum*, Hernandez-Viezcas *et al.*, (2011) in *Prosopis juliflora*, and Krishnaraj *et al.*, (2012) in *Bacopa monnieri*. For example, Tripathi *et al.*, (2015) in *Pisum sativum*, Hernandez-Viezcas *et al.*, (2011) in *Prosopis juliflora* and Krishnaraj *et al.*, (2012) in *Bacopa monnieri* all discovered elevated SOD, CAT, and POD activities induced by ZnO-NP in numerous plants. The plants were helped by these elevated antioxidant activities to scavenge the extra ROS produced in response to stressful stimuli.

**Biochemical characters:** ANOVA results on biochemical attributes (Phenolics, proline, Glycine Betain and Total Soluble Protein) on wheat varieties were presented in table 3 and 4. Means square results indicated that influence heavy metals plus ZnO NPs in biochemical attributes showed highly significant ( $p < 0.05$ ) results on all varieties of wheat at both years with highly significant interaction. Highest value of phenolic contents (mg/g f. wt) were obtained at ZnO (10, 20, 30ppm) application along with heavy metal (10ppm Cr) supplement in Faisalabad (2008) and less amount were detected in control and heavy metals application at both years (Fig. 5A). Maximum proline contents were indicated in Aas (2011) at both years at ZnO nanoparticles application in combination with heavy metals (Cr and As) and control and lowest at heavy metal stressed plants (Fig. 5B). Higher glycine butain concentration were obtained at T11 (30ppm ZnO + 10ppm Cr) at both varieties of wheat at both years and lowest contents were noted at control (Fig. 5C). Arsenic stress increased proline accumulation in soybean plants, according to studies by Choudhury *et al.*, (2011) in *Oryza sativa* and Ahmad *et al.*, (2020) in *Withania somnifera*. Proline and GB collection is increased, which protects biochemical procedures, improves ROS scavenging as well as preserves redox homeostasis and enzyme functioning (Jan *et al.*, 2020). Proline and GB, according to Samanta *et al.*, (2021), assist sustain antioxidant of plants and glyoxalase systems, boosting stress tolerance. According to Al Mahmud *et al.*, (2018), GB and GSH aid in metal chelation, which lowers the harmful consequences of metal stress. Significantly increase in protein contents were noted in ZnO 30 ppm + As 10 ppm in Faisalabad (2008) and decrease in Aas (2011) at ZnO 10 ppm at both years (Fig. 5D).

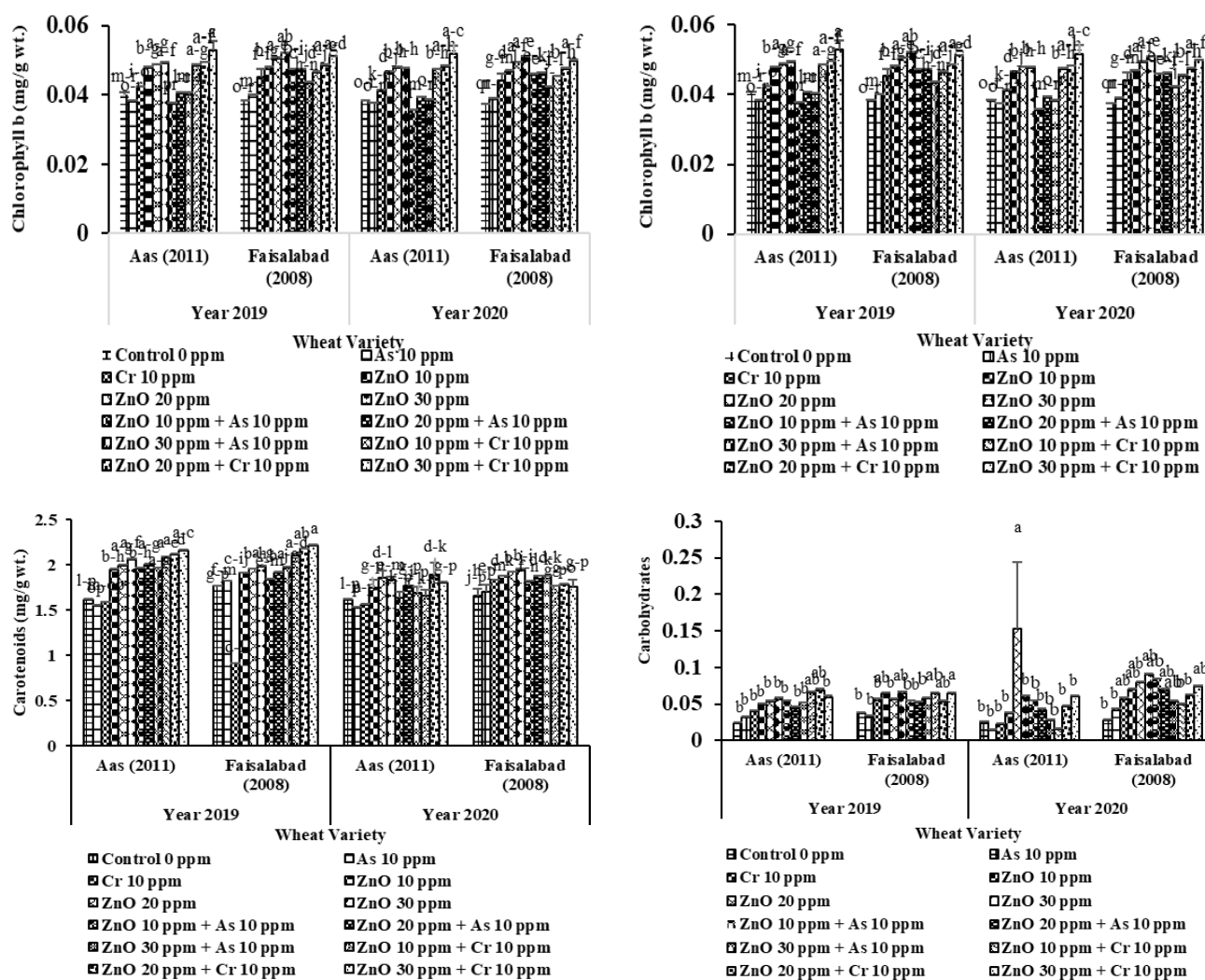


Fig. 3. Physio-chemical attributes of two different wheat varieties *Triticum aestivum* L. when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress. (A) Chlorophyll (a) mg/g, (B) Chlorophyll (b) mg/g, (C) Carotenoids mg/g weight, (D) Total Carbohydrate mg/g plant.

Table 3. Means squares (MS) from the analysis of variance (ANOVA) for antioxidant activities and biochemical attributes of two varieties of wheat (*Triticum aestivum* L.) when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress.

Source	df	MS of catalase activity (CAT)	MS of peroxidase activity (POD)	MS of superoxide-dismutase activity (SOD)	MS of carbohydrate contents	MS of phenolics contents	MS of proline contents
Variety	1	0.0854***	0.0941***	0.0077***	0.0070**	0.1184***	0.0034***
Treatment	11	0.0728***	0.0628***	0.0207***	0.0042***	0.0223***	0.0003***
Year	1	0.1676***	0.000063ns	0.0149***	0.00002ns	0.0028***	0.0018***
Variety × Treatment	11	0.0039***	0.0027***	0.0038***	0.00099ns	0.0008***	0.0001***
Variety × Year	1	0.0266***	0.0427***	0.2233***	0.00089ns	0.0054***	0.00002*
Treatment × Year	11	0.0091***	0.0036***	0.0024***	0.00211*	0.0008***	0.00001**
Variety × Treatment × Year	11	0.0008***	0.0025***	0.0022***	0.0008ns	0.0017***	0.00001***
Error	144	0.000030	0.000060	0.000021	0.000948	0.000023	0.000005
Total	191	1.23945	0.934993	0.570581	0.235338	0.413332	0.011782

Ns = Non-significant; \*, \*\*, \*\*\*, Significant at 0.05, 0.01 and 0.001, respectively

### ZnO analysis

**FTIR of ZnO nano particles:** The investigation of Zn-O nanoparticles in wheat plants exposed to heavy metal stress was displayed in Fig. 6A. The FTIR was employed to examine the structural bond found in the ZnO produced sample. ZnO nanoparticles were found to have FTIR spectra in the 4000-500  $\text{cm}^{-1}$  range. The peaks seen in the

500-1000  $\text{cm}^{-1}$  area were identified as the extended modes of ZnO nanoparticles. The peak at roughly 9705  $\text{cm}^{-1}$  was created by the hydroxyl group of water molecules that were absorbed. The peaks around 14415 and 15523  $\text{cm}^{-1}$  were produced by COC and OH bonds. The surface of the ZnO nanoparticles contains attached  $\text{H}_2\text{O}$  molecules, and this caused an exceptional peak seen at 317877  $\text{cm}^{-1}$  to be attributed to OH group extending vibration.

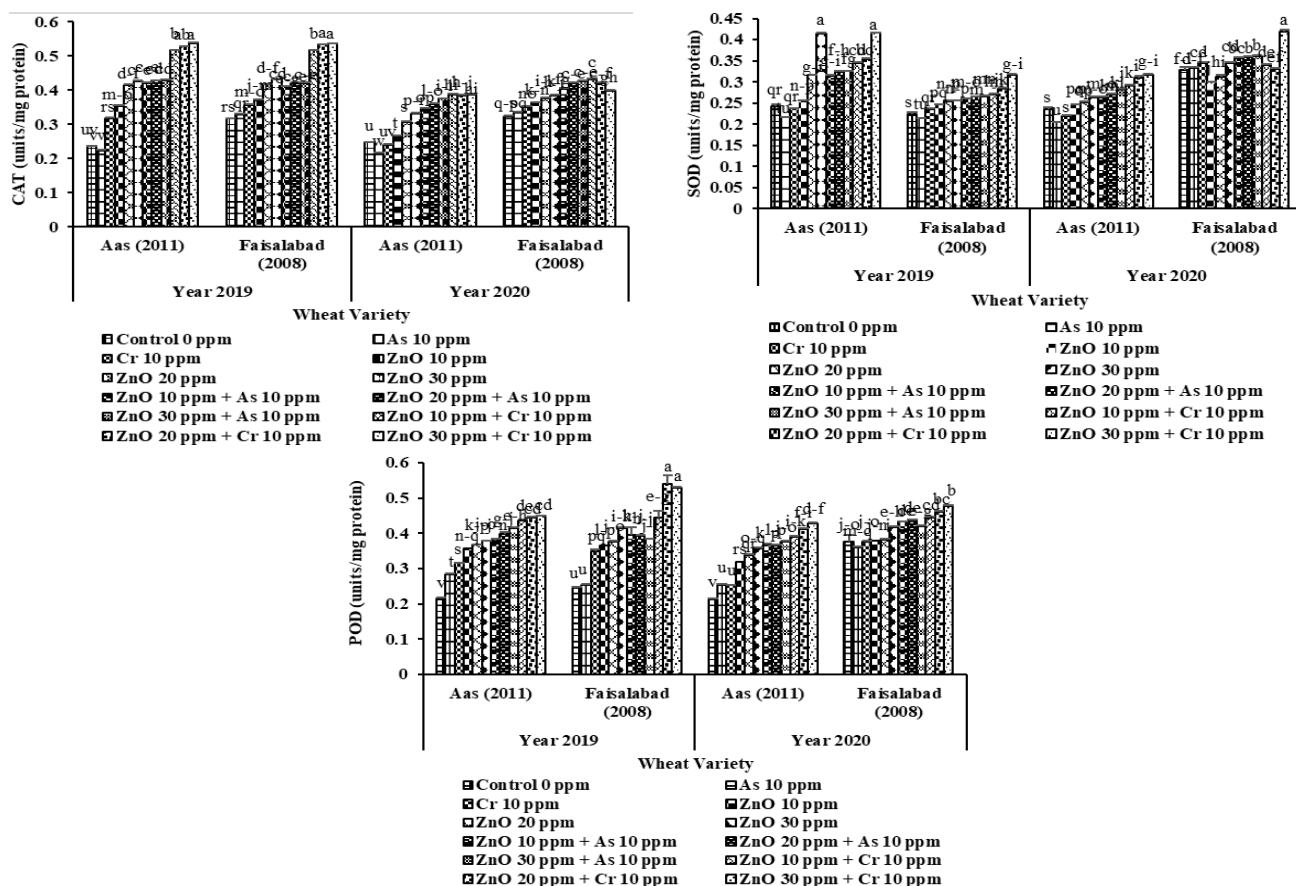


Fig. 4. Antioxidant activities of two varieties of wheat (*Triticum aestivum* L.) when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress. (A) CAT (Units/mg protein), (B) POD (Units/mg protein), (C) SOD (units/mg protein).

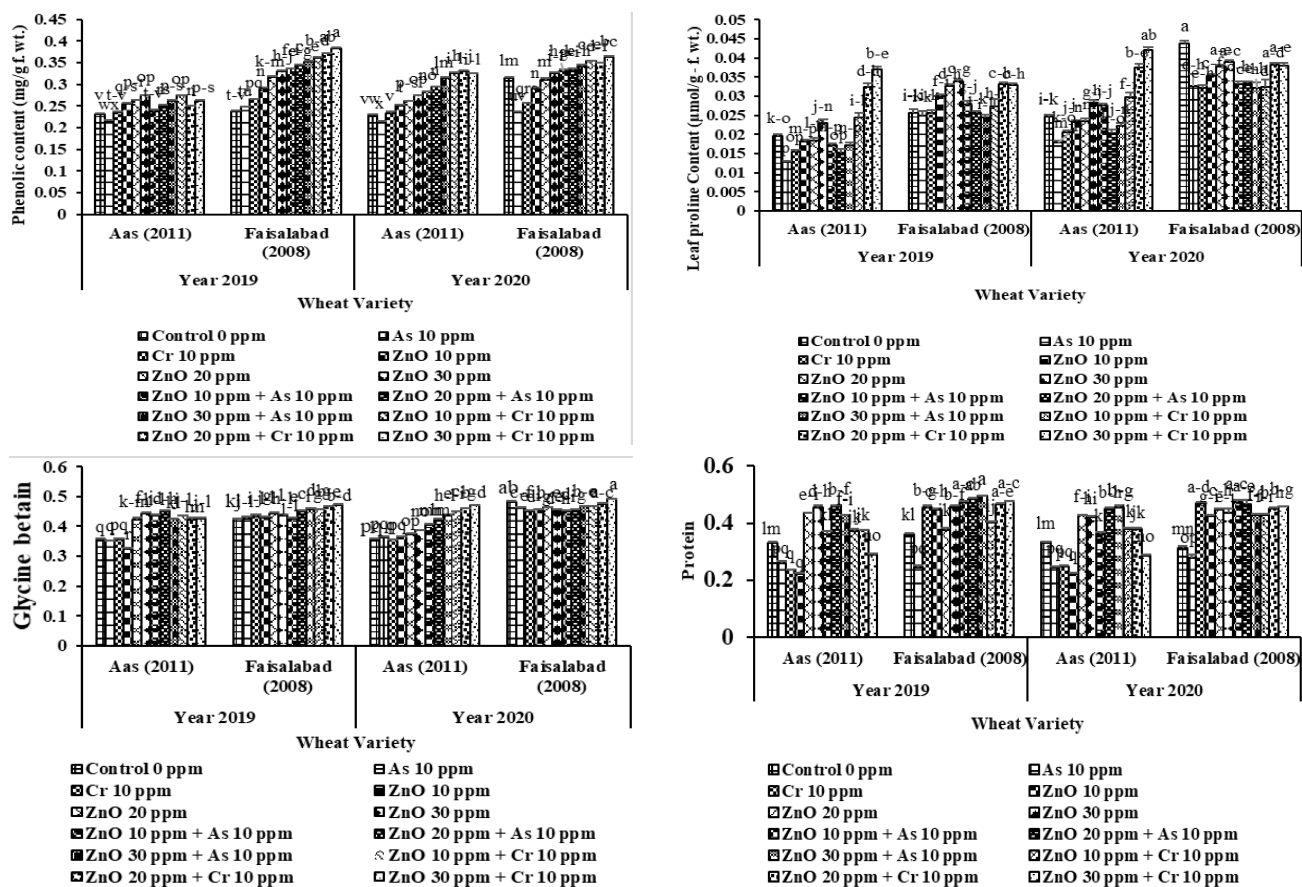


Fig. 5. Biochemical attributes of two different varieties of wheat (*Triticum aestivum* L.) when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress. (A) Total phenolic content (mg GAE/g f. wt.), (B) Leaf proline content (μmol/g - f. wt.), (C) Glycine betain, (D) Total soluble protein.

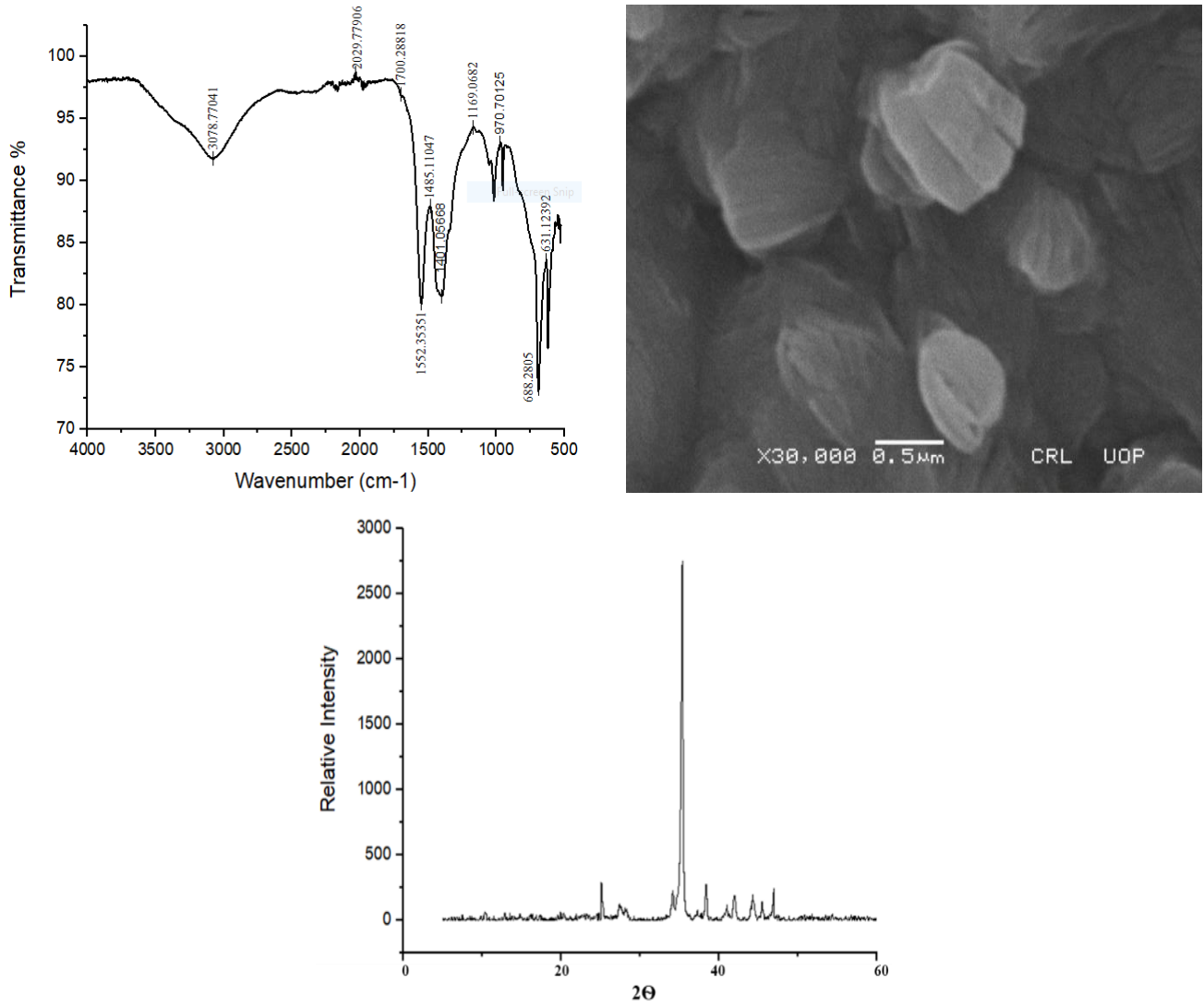


Fig. 6. Phycofabrication of zinc oxide nanoparticles (ZnONPs) using marine green alga *Ulva lactuca* extracts and characterized by: A) FTIR, B) FESEM, C) XRD analysis. (A) FTIR, (B) SEM of ZnO Nanoparticles, (C) XRD pattern of ZnO nanoparticles (Iqbal and Bhatti, 2021).

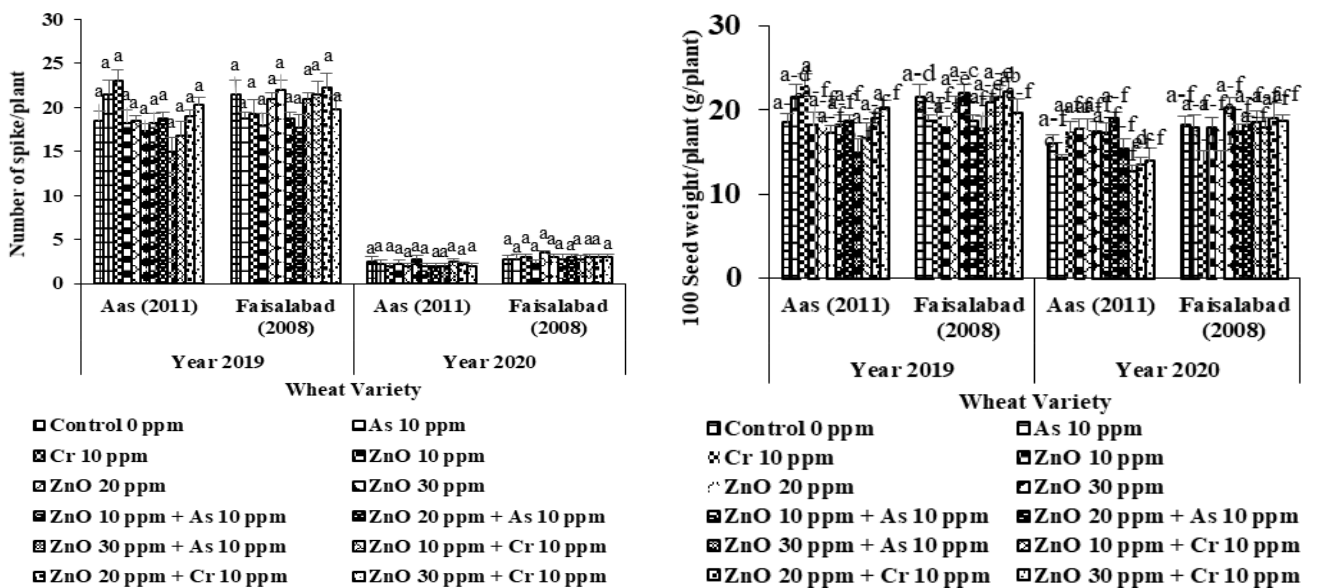


Fig. 7. Morphological attributes of two varieties of wheat (*Triticum aestivum* L.) when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress. (A) Number of spike/plant, (B) 100 Seed weight/plant (g/plant).



**SEM of ZnO nano particles:** The superficial morphology of a prepared sample of Zn-O nanoparticles was examined using scanning electron microscopy, as shown in Fig. 6B. The homogeneous and consistently dispersed ZnO nano particles were clearly visible in the microscopy photos.

**XRD analysis of Zn-O nano particles:** The structural phases and crystals of Zn-O nanoparticles generated with extract of Aloe vera were validated by X-ray diffraction analysis, and the pattern was reported in the 200–600 range, as shown in Fig. 6C. The lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202), respectively, are represented by the angles (200) of 25.30, 36.10, 37.50, 41.50, 42.30, 45.20, 46.10, and 47.20 in this design.

**Yield attributes:** Data presented in Table 4 shown the heavy metals effect and ZnO NPs on yield attributes of two varieties of wheat at both 2019 and 2020. Means squares results observed that response of heavy metals and ZnO NPs on weight of seed and spikes number of two wheat varieties showed non-significant ( $p>0.05$ ) results whereas response of varieties as well as years showed highly significant ( $p<0.05$ ) outcomes. Increase in yield might be due to enhanced photosynthesis activities which produced healthy biomass that also resulted better yield of wheat. These findings are similar to those of Rizwan *et al.*, (2017), who found that applying ZnO-NP to Cd-stressed maize plants significantly increased growth. In several other species, including *Leucaena leucocephala*, ZnO-NPs application has been shown to decreased oxidative stress caused by heavy metal (Venkatachalam *et al.*, 2017) (Fig. 7).

**Table 4. Means squares (MS) from the analysis of variance for the biochemical and yield parameters of two varieties of wheat when subjected to foliar treatment of ZnO nanoparticles under arsenic and chromium stress.**

Source	df	MS of glycine betain	MS of total soluble protein	MS of seed weight	MS of number of spike/plant
Variety	1	0.1196***	0.266***	1 09.505***	5.3333*
Treatment	11	0.0096***	0.660***	4.426ns	0.3049ns
Year	1	0.0051***	0.0005*	312.630 ***	16.3333***
Variety × Treatment	11	0.0042***	0.029***	28.062***	0.2992ns
Variety × Year	1	0.0053***	0.0003ns	0.880ns	6.0208**
Treatment × Year	11	0.0014***	0.0011***	14.323*	0.1629ns
Variety × Treatment × Year	11	0.0013***	0.0018***	11.573ns	0.1686ns
Error	144	0.00004	0.0001	7.686	0.7847
Total	191	0.3207	1.301	2171.99	150.979

Ns = Non-significant; \*, \*\*, \*\*\*, Significant at 0.05, 0.01 and 0.001, respectively

## Conclusion

It was concluded that the toxicity of heavy metals (As and Cr) in wheat plants was effectively decreased using different doses of ZnO-NPs. Therefore, the use of ZnO-NPs can be effective to cultivate wheat crop in heavy stressed soils. As a result, nanoparticles can enhance crop enactment via lowering toxicity of as and Cr.

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