ACS OMEGA

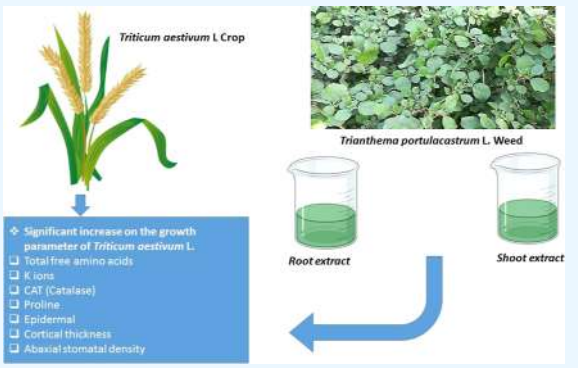
http://pubs.acs.org/journal/acsodf
Article

Morphoanatomical and Physiological Adaptations of *Triticum aestivum* L. against Allelopathic Extract of *Trianthema portulacastrum* L. (Horse purslane)

Muhammad Shahid Hassan, Nargis Naz, Habib Ali,* Basharat Ali, Muhammad Akram, Baber Ali, Faisal Mahmood, Umbreen Shahzad, Mumtaz Hussain, Rashid Iqbal,* Sezai Ercisli,* Mohamed Farouk Elsadek, Abd El-Zaher M. A. Mustafa, Ijaz Ahmad, and Reham M. Mostafa

Cite This: https://doi.org/10.1021/acsomega.3c03238

ABSTRACT: Weed infestation can be harmful to crop growth and cause severe losses in yield by absorbing nutrients and releasing inhibitory secondary metabolites and thus needs to be controlled for food security. The use of synthetic herbicides is one of the most widely applied methods, but its frequent usage is a serious threat to health and the environment and develops resistance in weeds. Allelopathy is an eco-friendly bio-control method, and *Trianthema portulacastrum* extracts are known to be effective against various weeds in the crop of *Triticum aestivum* (wheat), but their effect on the main crop (wheat) is still unknown. The pot experiment was carried out, and various concentrations (30, 60, and 100%) of root and shoot extracts of *T. portulacastrum* and a synthetic herbicide (Metafin Super) along with control (distilled water) were applied to the wheat plants. Various morphological, physiological, and anatomical parameters were recorded under natural conditions. The objective of this study was to explore the allelopathic impact of *T. portulacastrum* compared to the synthetic herbicide on the growth of wheat. This study displayed that various growth characteristics of wheat were significantly affected at $p \leq 0.05$ by root and shoot water extracts of *T. portulacastrum* but were less inhibitory as compared to the synthetic herbicide. This inhibition of the growth of wheat was coupled with a significant increase in total free amino acids, K ions, CAT (catalase), proline, epidermal and cortical thickness, and abaxial stomatal density. In addition, a reduction in growth parameters was correlated with a decrease in photosynthetic pigments. This study revealed that the use of *T. portulacastrum* extracts could be safer than synthetic herbicides for wheat plants and would be beneficial to control weeds in a wheat field.



1. INTRODUCTION

The world population is growing at an alarming rate, as it has dramatically doubled from that of 1970 and is estimated to reach 9.2 billion with a 30% annual growth by 2050.[1] A massive increase in population leads to food scarcity, resulting in 70% increase in food demand globally.[2,3] Wheat is among the most pre-historic and widely cultivated crops of all cereals.[4] This is a key source of carbohydrates (60−90%), proteins (11−16.5%), lipids (1.5−2%), inorganic essential ions (1.2−2%), and vitamins[5] for both animals and human beings. In Pakistan, it was cultivated in an area of 9.593 Mha, and the yield was about 27.10 million tons in season 2020−21. To make up for nutrition deficiency, the yield can be improved through the employment of high-yielding genotypes coupled with advanced weed-controlling methods.[6] The weather conditions and the soil of Pakistan are suitable for wheat production, but the wheat yield is 10 times lesser than that of developed countries.[7]

Several biotic and abiotic factors cause low wheat yield;[8,9] among these, weed infestation is one of the main limiting biological constraints.[10] Almost 30,000 weed species have been recognized in the world, and out of these, 18,000 species can cause a significant loss to crop yield.[11] They are the main bottleneck in crop-growing systems as they compete for space, capture nutritional precious resources, and ultimately lead to a significant decrease in wheat crop production. The yield losses of wheat vary between 17 and 30% annually[12] depending on the density and flora of weed. Therefore, the management of weeds is a basic requisite for better productivity.[13] Various

Received: May 19, 2023
Accepted: July 27, 2023

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

strategies that are most commonly employed for weed control are mechanical, physical, chemical, and cultural practices. Currently, many herbicides are being used extensively to limit the growth of weeds in important crops;[14] unfortunately, they lead to severe health and environmental issues,[15] and moreover, synthetic herbicides pose negative effects on wheat crop. Metribuzin and isoproturon with diflufenican reduced plant length due to the toxic effect.[16] Chlorotoluron, a phenyl urea herbicide, induced oxidative stress in wheat,[17] while isoproturon caused a significant decrease in chlorophyll content and ultimately reduced the shoot length and dry weight of the wheat plants.[18]

There is now a need to try bio-herbicides as alternatives to synthetic herbicides for weed management. Therefore, plant products are gaining the interest of investigators due to their safe and eco-friendly nature. Over the last few decades, the trend to comprehend the trend of allelopathy has been receiving more interest among researchers. Allelochemicals are usually secondary metabolites[19,20] and are released in the environment by various parts (leaves, flowers, roots, seeds, and stems), by decomposition, or by leaching from plant residues, mainly terpenoids, cyanides, phenolics, and fatty acids.[21] These phytochemicals alter the biochemical responses, and as a result, the physiological process undergoes modification. The mode of action of these allelochemicals is similar to commercial herbicides, so are considered bio-herbicides.[22] Morphological and anatomical changes are a result of the modification in the physiological processes of the victim plant. These changes can result in hormonal imbalance, enzyme activity, protein synthesis, photosynthesis, and respiration through allelochemicals.[23]

Various studies have been reported on the allelopathic effect on wheat. Nasira et al.[24] calculated that the powder of *Fumaria indica* and *Asphodelus tenuifolius* reduced the plant height shoot fresh and dry weight of the wheat plant. Muhammad and Majeed[25] studied that sunflower allelopathic extracts inhibited the growth of wheat at an early stage. Hameed et al.[26] studied the morphological and physiological inhibition responses of wheat varieties against the leaf extract of *Alstonia scholaris* (L.). *Trianthema portulacastrum* is a summer weed and is known for its allelopathic effects, most probably due to its numerous active compounds such as phenols, terpenoids, alkaloids, caffeic acid, vanillic acid, *trans*-cinnamic acid, ferulic acid, *o*-coumaric acid, pyrogallic acid, and protocatechuic acid.[27] Recently, we have reported that *T. portulacastrum* suppressed the growth of *Convolvulus arvensis* L. and *A. tenuifolius* which are drastic weeds primarily found in wheat crop. *T. portulacastrum* extracts are known to be effective against weeds in wheat,[19,28] but their inhibitory or stimulatory effects on wheat were still unknown. The objective of the current study was to explore the allelopathic impact of *T. portulacastrum* compared to the synthetic herbicide (Metafin Super) on the growth of wheat (crop) to assess its use as a bio-herbicide on wheat crop weeds.

2. MATERIALS AND METHODS

The experiment was accomplished to estimate the allelopathic influence of *T. portulacastrum* on the various growth parameters of wheat.

2.1. Collection of Allelopathic Plants and Formation of an Aqueous Extract. *T. portulacastrum* plants were collected from the cotton fields of Layyah (30.9693°N, 70.9428°E) in July 2017 (Figure 1). Whole plants were

Figure 1. *T. portulacastrum* L. weed used in root and shoot extract treatments.

washed thoroughly with distilled water and dried under shade. After the separation of roots and shoots, these dried samples were ground with the help of a grinder into a fine powder separately and were kept in clean and sealed glass containers at room temperature till their use for study. The aqueous extract was prepared by soaking 10 g of powder of each part of *T. portulacastrum* separately in 100 mL Of distilled water (10% w/v) for 24 h at room temperature. Each part extract was filtered by Whatman no. 1 filter paper and considered 100%. The extract-filtered solutions were used to give final concentrations of 30 and 60% of each part.

2.2. Experimental Design. A pot experiment was carried out at the botanical garden of the Islamia University of Bahawalpur, Punjab, Pakistan (29.3783°N and 71.7647°E). The seeds of *Triticum aestivum* (cultivar Sahar-2006) were purchased from an agricultural crop shop. The pot experiment was carried out in a completely randomized design with three replicates. 15 healthy wheat seeds were sown in each pot (having 25 cm height and 10.5 cm width with 5 kg of garden soil capacity) during December 2017 and then repeated during 2018 after imbibition of seeds in cold water for 24 h. The soil used in the pots was analyzed with physicochemical characteristics such as pH (8.1) by a pH meter, electrical conductivity (1.97 ds/m) by an EC meter, and available K (113 ppm), P (6 ppm), and organic matter (0.51%) along with texture (Sandy loam) according to Handbook no. 60.[29] The pots were regularly irrigated with tap water to maintain the moisture content of the soil. After the germination had been completed, 10 healthy seedlings were left in each pot. The pots were arranged into eight groups with three replication and labeled T1, T2, T3, T4, T5, T6, T7, and T8 (Figure 2). Distilled water to the T1 group, 30% of 10 mL root extract to the T2 group, 60% of 10 mL root extract to the T3 group, 100% of 10 mL root extract to T4, 30% of 10 mL shoot extract to the T5 group, 60% of 10 mL shoot extract to the T6 group, 100% of 10 mL shoot extract to the T7 group, and 10 mL of herbicides

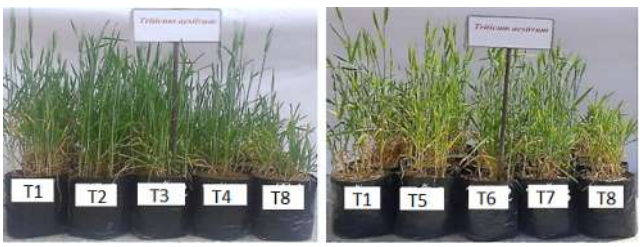


Figure 2. Pot experiment of root and shoot water extract treatment of *T. portulacastrum* and herbicide on the wheat.

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

(metsulfuron 14.3% + tribenuron methyl 14.3%) to the T8 group were applied through the soil. Each treatment has three replicates, and each replicate has 10 crop plants in the pot. After 2 months of sowing seeds in pots, 10 mL of aqueous extracts of each level of *T. portulacastrum* and herbicide solution were applied two times with a 1 week gap to the respective pot's soil.

2.2.1. Assessments. This data was collected after 30 days of the application of the allelopathic and herbicide treatments to study the shoot length (cm), root length (cm), leaf area (cm^2), shoot dry weight (g/plant), root dry weight (g/plant), and a number of seeds per plant.

The following physicochemical characteristics were studied from the wheat samples:

2.2.1.1. Chlorophyll Estimation (mg/g). Chlorophyll *a* and *b* levels in wheat were estimated by Arnon's method.[30] The leaf samples from each treatment were soaked in an acetone solution. Pigment absorbance was computed at 645 and 663 nm wavelengths by using an ELISA plate.

$$\text{Chl. } a \text{ (mg/g f. wt)} = (12.7 \times \text{A } 663 - 2.69 \times \text{A } 645) \times V/1000 \times W)$$

$$\text{Chl. } b \text{ (mg/g f. wt)} = (22.9 \times \text{A } 645 - 4.68 \times \text{A } 663 \times V/1000 \times W)$$

2.2.1.2. Proline Determination (mg/g). The proline amount was recorded.[31] Fresh leaf samples (0.5 g) were disrupted in 10 cm^3 of 3% sulfosalicylic acid. After filtration of the homogenous mixtures of each sample was mixed with an acidic ninhydrin solution, phosphoric acid, and acetic acid. Then, toluene with chromophore was heated, and the absorbance was noted at 520 nm.

2.2.1.3. Determination of Catalase (U/mg). The catalase activity was recorded as the consumption level of H_2O_2 by the enzyme and converted into H_2O and O_2 by the method of Chance and Maehly.[32] The absorbance was measured at 240 nm on an ELISA plate every 20 s.

2.2.1.4. Determination of K Ions and Total Free Amino Acids (mg/g d.wt). The content of potassium ions was calculated by a flame photometer (Jenway, PFP-7) according to Kacar.[33] The potassium ion values with a flame photometer were determined by comparison with standard curves, while total free amino acids (TFA) in the tested sample were determined by Hamilton and Van Slyke.[34] The optical density of the sample solution was recorded at 570 nm by a spectrophotometer.

For the anatomical study, wheat stem and leaf samples were prepared by killing and fixing in F.A.A. (10 mL of formalin, 5 mL of acetic acid, and 35 mL of ethyl alcohol 95%). Freehand sectioning slides of various parts of wheat specimens were prepared, double-stained dehydration with safranin and fast green, cleaned in xylene, and mounted in Canada balsam and studied various cells and tissues of stems and leaves. The prepared slides were analyzed microscopically, and measurements were taken with an ocular micrometer. Photomicrography with a digital camera equipped with a Nikon stereomicroscope (Nikon 104, Japan) was taken. Different anatomical parameters of stems (epidermal thickness, cortex thickness, metaxylem cell area, phloem thickness, vascular bundle area, and pith area) and leaves (upper epidermal thickness, lamina thickness, phloem thickness, adaxial stomatal density, and abaxial stomatal density) were studied.

2.3. Statistical Analysis. The analysis of variance (ANOVA) and LSD test were applied by using the software "Statistix version 8.1". Pearson's correlation was calculated by "Microsoft Excel" and "Statistix version 8.1" between the morphology, physiology, and anatomy of wheat.

3. RESULTS

3.1. Morphological Characteristics. The results (Figures 3 and 4) illustrated different responses in various morphological characteristics of wheat with increasing allelopathic levels of root and shoot extracts of *T. portulacastrum*. Most of the growth parameters showed a declining pattern. However, the shoot length was reduced in the extract compared to control, and the shoot dry weight showed a slight increase at the diluted root and shoot extracts, while the root length only increased at the diluted level of the root extract. However, herbicide treatment caused more decline than the root and shoot extracts in all morphological characteristics of wheat.

Figure 3. Effect of root and shoot water extracts of *T. portulacastrum* and herbicide on the morphological parameters of wheat (T1 = distilled water; T2 = 30% root extract; T3 = 60% root extract; T4 = 100% root extract; T5 = 30% shoot extract; T6 = 60% shoot extract; T7 = 100% shoot extract; and T8 = herbicide treatment).

3.2. Physiological Characteristics. The chlorophyll *a* and *b* levels of wheat were constantly and gradually decreased with increasing root and shoot extract levels. However, the chlorophyll level increased somewhat at 30% root extract of *T. portulacastrum,* and the chlorophyll *b* content increased at 30% of shoot extract. The maximum decline was recorded in both pigments at herbicide treatment. The catalase (CAT) activity of *T. aestivum* increased in all levels of root and shoot extracts (Table 1).

The application of both extracts of *T. portulacastrum* illustrated a non-significant increase in the proline level of wheat. A gradual increasing trend was observed in the proline level with increasing levels of both extracts. The highest value of proline was recorded at herbicide treatment (Table 1). The gradual increasing trend in the TFA level was calculated with increasing root extract and shoot extract levels. Meanwhile, herbicide treatment also caused a significant increase in this parameter (Table 1).

3.3. Anatomical Characteristics. *3.3.1. Stem Anatomy.* The results in Table 1 show that the epidermis thickness and cortical thickness of wheat increased significantly at all treatments of root and shoot extracts of *T. portulacastrum* as

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

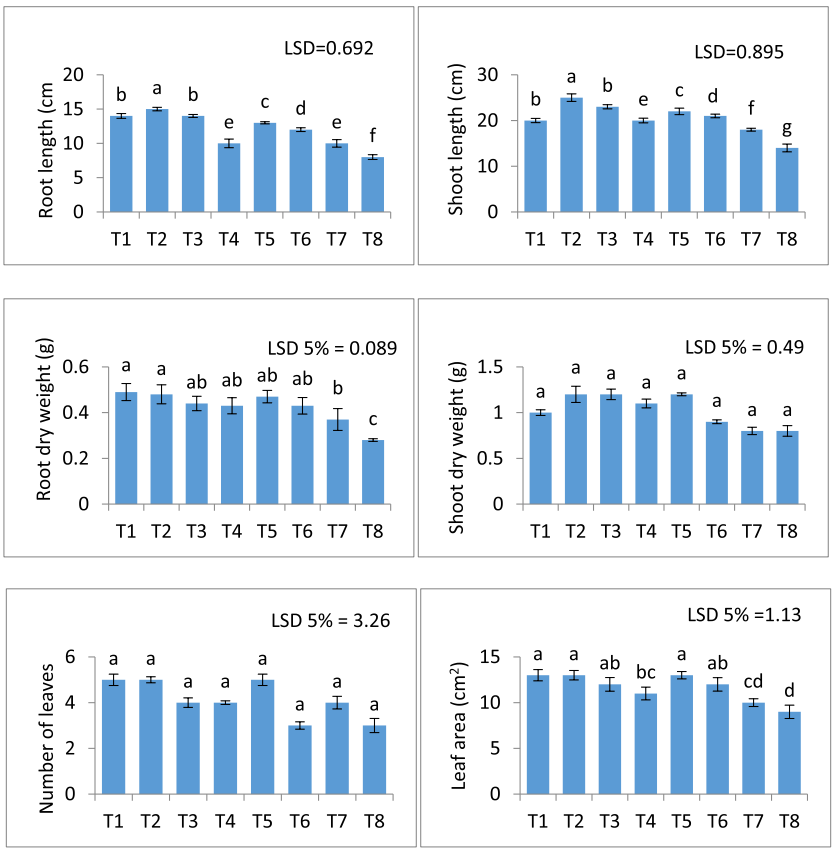


Figure 4. Effect of root and shoot water extracts of *T. portulacastrum* and herbicide on the morphological parameters of wheat (T1 = distilled water; T2 = 30% root extract; T3 = 60% root extract; T4 = 100% root extract; T5 = 30% shoot extract; T6 = 60% shoot extract; T7 = 100% shoot extract; and T8 = herbicide treatment).

Table 1. Effect of Root and Shoot Water Extracts of *T. portulacastrum* and Herbicide on the Physiological Characteristics of Wheat[a]

treatment groups	T1	T2	T3	T4	T5	T6	T7	T8	LSD	CV	GM	*F* value
						Physiology						
chl. *a* (mg/g)	1.5 a	1.55 a	1.25 bc	1.21 bc	1.45 ab	1.2 bc	1.1 c	1.1 c	0.27	12.34	1.28	3.9*
chl. *b* (mg/g)	1.1 a	1.1 a	0.93 b	0.88 bc	1.2 a	0.95 b	0.85 bc	0.82 c	0.11	6.49	0.98	14.1***
CAT (U/mg)	18 g	26 ab	27 a	24 cd	22 e	20 f	23 de	25 bc	1.06	2.64	23.12	74.4***
proline (mg/g)	0.1 c	0.13 c	0.16 bc	0.2 abc	0.18 abc	0.22 abc	0.26 ab	0.29 a	0.13	38.97	0.19	2.3^{NS}
TFA (mg/g)	4 e	5 d	5 d	7 b	6 c	7.6 ab	8.2 a	7.2 b	0.74	6.88	6.25	35.5***
K ions (mg/g)	14 g	21 f	28 d	28 d	25 e	34 b	30 c	35 a	0.63	1.35	26.87	1087***
						Stem						
ET (μm)	0.3 a	0.2 b	0.3 a	0.32 a	0.31 a	0.34 a	0.36 a	0.37 a	0.092	16.96	0.313	2.94*
CT (μm)	1 d	1.2 cd	1.5 bc	1.6 abc	1 d	1.3 cd	1.9 ab	2 a	0.490	19.68	1.44	5.46**
PT (μm)	1 ab	0.7 b	0.8 ab	1.2 a	0.8 ab	0.8 ab	0.9 ab	1.1 ab	0.424	26.84	0.912	1.49^{NS}
MXCA (μm^2)	0.9 a	0.9 a	0.5 b	0.4 bc	0.8 a	0.3 cd	0.3 cd	0.2 d	0.169	18.17	0.537	26.0***
VBA (μm^2)	8 bc	8 bc	7 c	7 c	9 b	9 b	11 a	12 a	1.010	6.58	8.87	28.7***
PiA (μm^2)	12 c	13 b	13 b	15 a	10 d	9 e	7 f	5 g	0.994	5.47	10.50	104***
						Leaf						
UET (μm)	0.4 cd	0.37 cd	0.44 bcd	0.51 ab	0.42 cd	0.47 abc	0.54 a	0.55 a	0.088	10.95	0.462	5.14**
LT (μm)	6 ab	5.7 ab	6.5 a	6.5 a	5.8 ab	5.5 b	5.5 b	5.6 b	0.803	7.88	5.89	2.37^{NS}
MXCA (μm^2)	0.4 a	0.37 ab	0.35 ab	0.35 ab	0.2 c	0.26 bc	0.34 ab	0.34 ab	0.138	24.52	0.326	1.95^{NS}
AdSD	75 a	50 g	58 e	65 c	48 h	55 f	63 d	70 b	1.210	1.160	60.50	552***
AbSD	90 d	90 d	80 e	60 g	95 c	100 b	105 a	75 f	1.180	0.790	86.97	1372***

[a]T1 = distilled water; T2 = 30% root extract; T3 = 60% root extract; T4 = 100% root extract; T5 = 30% shoot extract; T6 = 60% shoot extract; T7 = 100% shoot extract; and T8 = herbicide treatment. The treatments exhibit dissimilar letters within rows that represent significance ($p \leq 0.05$) level.

compared to the control. However, at 30% root water extract treatment, the epidermis thickness decreased considerably, while 60% root extract did not cause any change in epidermal thickness. The epidermis thickness and cortical thickness were

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

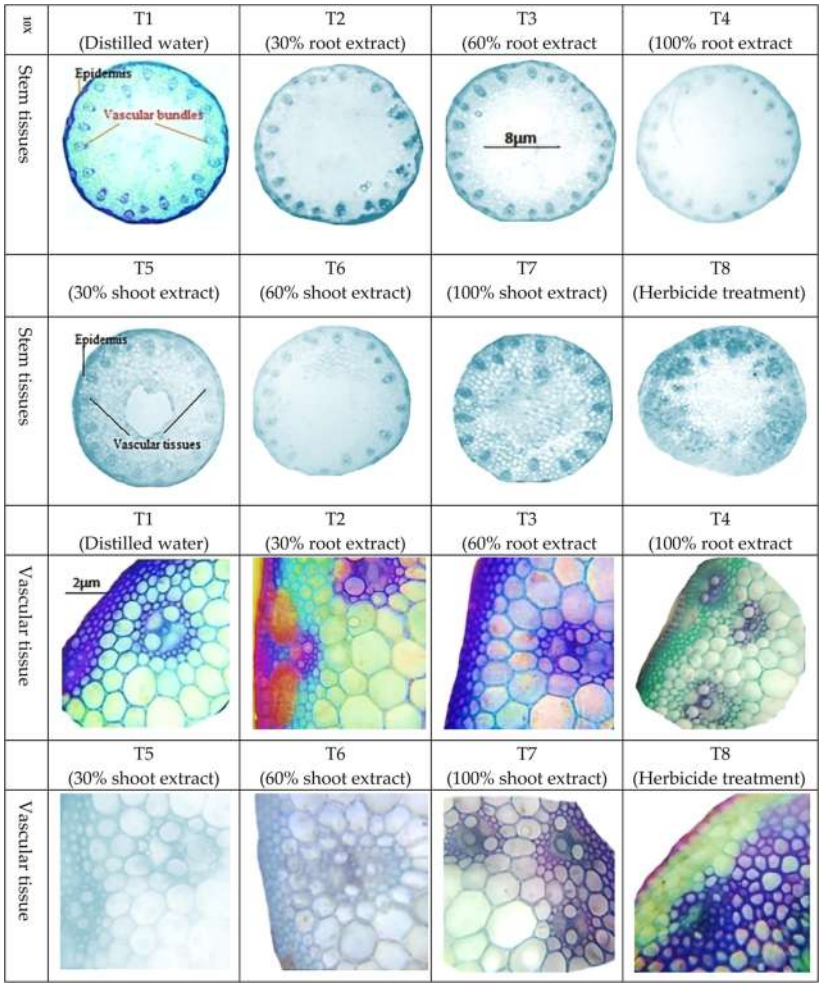


Figure 5. Stem anatomy of wheat by applying diverse levels of root and shoot extracts of *T. portulacastrum* and herbicide.

increased maximum at herbicide treatment (Table 1 and Figure 5). The phloem thickness and vascular bundle area of wheat treated with the extract of *T. portulacastrum* displayed a non-significant reduction trend at all treatments. However, root extract caused an increase in phloem thickness except for diluted levels. Meanwhile, herbicide treatment also indicated a slight increase in these parameters as compared to the control (Table 1 and Figure 5). Wheat treated with shoot and root aqueous extracts of *T. portulacastrum* revealed a significant reduction in metaxylem cell area as compared to the control. However, with herbicide treatment, the metaxylem cell area also decreased to a maximum (Table 1 and Figure 5). The root extract of *T. portulacastrum* promoted a slight and gradual increase in the pith area with an increasing level of concentration, while in the case of shoot water extract application, there was a gradual reduction in pith area at increasing levels. The maximum decline in pith area at the herbicide was recorded (Table 1 and Figure 5).

3.3.2. Leaf Anatomy. The results displayed that there was a slight reduction in the upper epidermal thickness, lamina thickness, and metaxylem cell area of wheat at extract treatments of *T. portulacastrum*. However, there was a slight increase in upper epidermal thickness at concentrated root extract and at all shoot extracts. Herbicide treatment also exhibited a significant increase in upper epidermal thickness and decrease in metaxylem cell area and lamina thickness (Table 1 and Figure 6). The root water extract levels of *T. portulacastrum* declined the adaxial and abaxial stomatal density in wheat. However, 30% root extract caused no variation in abaxial stomatal density. At shoot extract treatments, abaxial stomatal density increased. However, slight reduction was calculated in the adaxial and abaxial stomatal density in the case of treatment with herbicide (Table 1 and Figure 6).

3.4. Correlations between Morphological and Physiological Characteristics. In wheat root length, shoot length and root dry weight indicated a significant and positive correlation with the number of leaves, leaf area, shoot dry weight, chlorophyll *a*, and chlorophyll *b*. Meanwhile, chlorophyll *a* showed a significant and positive correlation with chlorophyll *b*, TFA with proline, and potassium with proline, while root length, shoot length, root dry weight, leaf area, number of leaves, chlorophyll *a*, and chlorophyll *b* indicated significant and negative correlations with proline, TFA and potassium ions. Similarly, leaf area also indicated a significant and negative correlation with proline and potassium ions (Table 2).

4. DISCUSSION

There are increasing weed management problems in crops due to the application of synthetic herbicides with increased environmental deterioration and also negative impacts on human health.[35] Among integrated weed control, allelopathy is

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

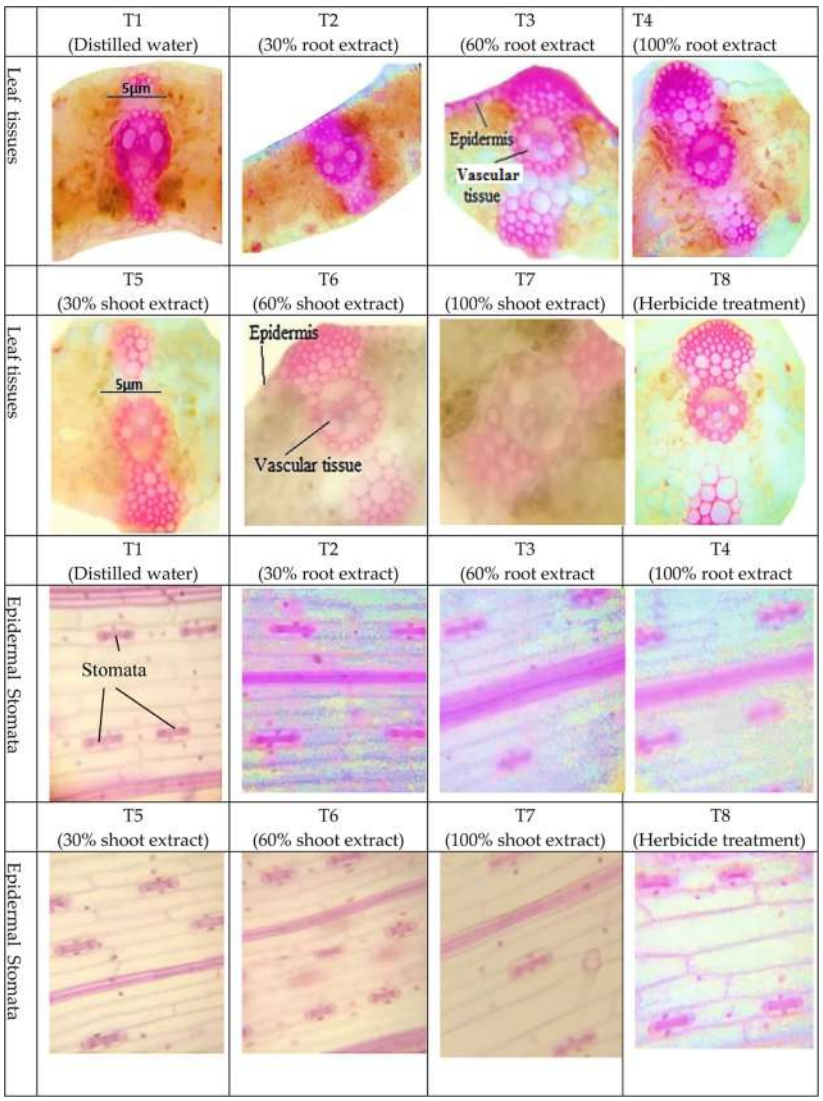


Figure 6. Leaf anatomy of wheat by applying diverse levels of root and shoot extracts of *T. portulacastrum* and herbicide.

Table 2. Effect of Root and Shoot Water Extracts of *T. portulacastrum* and Herbicide on the Correlations between Morphological and Physiological Characteristics of Wheat[a]

	RL	SL	RDW	SDW	NL	LA	a	b	CAT	Proline	TFA	K
RL	1.00											
SL	0.89	1.00										
RDW	0.88	0.86	1.00									
SDW	0.73	0.84	0.74	1.00								
NL	0.70	0.60	0.76	0.66	1.00							
LA	0.93	0.86	0.96	0.74	0.72	1.00						
a	0.84	0.70	0.82	0.69	0.86	0.89	1.00					
b	0.77	0.65	0.80	0.65	0.81	0.89	0.92	1.00				
CAT	-0.08	0.14	-0.30	0.31	-0.12	-0.30	-0.21	-0.34	1.00			
Proline	-0.90	-0.76	-0.91	-0.72	-0.78	-0.89	-0.88	-0.74	0.20	1.00		
TFA	-0.80	-0.54	-0.67	-0.66	-0.70	-0.72	-0.82	-0.65	0.04	0.90	1.00	
K	-0.71	-0.48	-0.76	-0.49	-0.89	-0.72	-0.86	-0.71	0.33	0.89	0.83	1

Significant Level	Positive Correlation	Negative Correlation
0.05		
0.01		
0.001		

[a]T1 = distilled water; T2 = 30% root extract; T3 = 60% root extract; T4 = 100% root extract; T5 = 30% shoot extract; T6 = 60% shoot extract; T7 = 100% shoot extract; and T8 = herbicide treatment.

an eco-friendly technique. Our major finding is that the extracts of *T. portulacastrum* are less toxic for wheat than synthetic herbicides. Analogous to the present results, sorghum and sunflower extract application greatly suppressed the root and shoot length of weeds.[36] Studies were carried out by Hamidi and Ghadiri[26] to judge the influence of wild barley

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

water extracts on wheat with a result of a diminution in shoot and root length. We have observed that the shoot extract caused more retardation in the wheat growth parameters than the root extract, so it was more allelopathic than the root extract but herbicide caused maximum suppression in wheat plants. Similar results were reported by Asghar et al.,[37] who calculated that different extracts of *T. portulacastrum* and *S. portulacastrum* repressed the shoot length significantly in *Trigonella foenum-graecum*. Inhibition in shoot length in weed species was also reported by Kandhro et al.,[36] who found that water extract powders of sorghum and sunflower are associated with the presence of certain allelopathic compounds with phytotoxic effects. Shoot length decline in the current study can be attributed to amendment in nucleic acid replication, mitochondrial metabolic process, and modification in mitosis.[38]

Quite a lot of studies have perceived that several phytochemicals are incorporated into the environment, either as exudation from living plant parts or by the decay of the plant material. These metabolites (terpenoids, phenolics, alkaloids, and their derivatives) are potent inhibitors of fresh weight and dry weight of neighboring plants,[39] and this is probably the case in our findings of reduced fresh and dry weight in wheat. Our results are also in accordance with Ghimire et al.,[40] who reported that the allelochemicals of *Miscanthus sacchariflorus* reduced the dry weight of weeds. The decrease in dry weight with increasing concentration of extracts was also described by Jafariehyazdi and Javidfar,[41] who concluded that the decline in the dry biomass of tested weeds may be linked to the decrease in root and shoot length caused by allelopathic compounds present in aqueous extracts of *T. portulacastrum*. The findings of the present work are reinforced by Naeem et al.,[42] who depicted a decline in the dry weight of weed with the application of sorghum and sunflower extracts. Application of plant water extract from sorghum, sunflower, and mulberry caused 88% inhibition in the total dry weight of weed plants.[42] A decrease in dry weight may be linked with a reduction in enzyme activity as a result of a decline in the biosynthesis of materials. The above-mentioned discussion describes the possible mechanisms of the reduced inhibitory effect of *T. portulacastrum* extracts on wheat plants.

The results of our study correspond to Zohaib et al.,[43] who described that *M. parviflora* and *C. murale* aqueous extracts of different levels posed a negative effect on a number of leaves of barley. Ramgunde and Chaturvedi[44] described a declining trend in the leaf area of *M. capitata* by application of leaf leachates of *Ricinus communis* and *Vitex negundo*. This diminution in leaf area might be due to the inhibition potential of allelochemicals in the biosynthesis of growth hormones like auxins, gibberellins, and cytokinins, consequently mutilation in metabolic processes. Hence, synthetic herbicides exhibited more drastic effects on wheat than *T. portulacastrum* extracts probably via enhanced leaf area reduction mechanisms. More reduction in the pith area in the wheat stem at herbicide treatment was also described by Mahakhode et al.,[45] who reported the demolition of pith cells under herbicide application. A slight increase in epidermal thickness in wheat stem was supported by Akram et al.,[46] who reported the increase of thickness in *Capparis decidua* in highly saline areas. A decrease in metaxylem area in wheat in most of the levels of root and shoot extracts in the present study leads to an increase in sap conduction ability because narrow xylem vessels provide more capillarity action for upward movement of water.[47] This increase in stomatal density in the present research was supported by Al-maskri et al.[48] and Lei et al.[49] They reported a noteworthy increase in the number of stomata in *T. aestivum* leaves affected by various types of stresses. Hence, these adaptations suggest higher damage to wheat plants under synthetic herbicide treatment. Allelochemicals released from weeds demonstrate a decline in the growth of crop plants by intervention in fundamental physiological activities. Moreover, proline and other cell solutes regulate osmotic balance under stress situations.[50−52] Allelochemicals of the root and shoot of *T. portulacastrum* in the present study interacted with the various physiological characteristics of wheat in a less damaging manner than synthetic herbicides. Chlorophyll has an imperative role in photosynthesis and represents a key indicator for tolerance investigation against stress.[53−58] Mabasa et al.[59] described the depletion of chlorophyll content in two species of bean under plant extract and herbicide treatments. Water extracts of *T. portulacastrum* reduced the levels of chlorophylls *a* and *b* in weed species.[60] The chlorophyll pigments constantly declined in the current research with an increase in the levels of *T. portulacastrum* residues because phytochemicals negatively influence the rate of chlorophyll synthesis and less availability of water and minerals. Hence, the lesser reduction of pigments in wheat under *T. portulacastrum* extracts is likely due to the above-mentioned mechanisms.

Allelochemicals of *T. portulacastrum* root and shoot had mostly promoted the catalase enzyme and TFA in wheat plants compared to synthetic herbicides in the present work (Table 1). El-Shora[61] calculated such kind of enhancement activity in chickpea after treatment with the root extract of *Rumex dentatus*. A plant's oxidative stress may be compensated with the regulation of antioxidants including catalase and peroxidase.[62−64] Catalase serves as the prime defense in the detoxification of hydrogen peroxide.[65−68] Analogous to our work, synthetic herbicides enhanced the catalase level in cereal leaves.[68] Plants regulate the activity of antioxidant enzymes like catalase at higher levels of stress to degrade ROS (reactive oxygen species) due to stop oxidative stress.[69−71] A high level of free amino acids under *T. portulacastrum* extract is presumed to be the elevating protein content which is due to a high decline in the protease activity during stress that is very significant for the hydrolysis of reserve proteins.[72] Analogous to our work, Maqbool et al.[73] described an enhanced amount of free amino acids in corn plants under biotic stress. Thus, we consider that the enhanced catalase enzyme activity and TFA content in wheat plants under *T. portulacastrum* extracts, compared to the synthetic herbicide, are most likely reasons for less inhibitory effect in wheat.

5. CONCLUSIONS

A minimum significant decline was observed in chlorophyll contents, leaf area, plant height, and dry weight of wheat under the treatment of extracts of *T. portulacastrum* compared to the synthetic herbicide treatment, which confirms that it is relatively safer to be used as a bio-pesticide. The changes in anatomical parameters in wheat plants under herbicide treatment suggest higher damage compared to *T. portulacastrum* extracts. However, diluted concentrations of allelopathic extract of *T. portulacastrum* may act as a growth promoter, and high concentrations suppress the growth of the root, stem, and leaves. Enhanced catalase activity and TFA content in wheat plants under *T. portulacastrum* extracts, compared to the

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

synthetic herbicide, are most likely reasons for less inhibitory effects in wheat. Finally, the use of *T. portulacastrum* extracts would be safer than synthetic herbicides for wheat plants and would be beneficial to control weeds in a wheat field.

AUTHOR INFORMATION

Corresponding Authors

Habib Ali − *Department of Agricultural Engineering, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan 64200, Pakistan*; Email: habib_ali@yahoo.com

Rashid Iqbal − *Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan; Department of Agroecology-Climate and Water, Aarhus University, Tjele 8830, Denmark*; Email: rashid.iqbal@iub.edu.pk

Sezai Ercisli − *Department of Horticulture, Agricultural Faculty, Ataturk Universitesi, Erzurum TR 25240, Turkiye; HGF Agro, Ata Teknokent, Erzurum TR-25240, Turkiye*; Email: sercisli@atauni.edu.tr

Authors

Muhammad Shahid Hassan − *Department of Botany, The Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan*

Nargis Naz − *Department of Botany, The Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan*

Basharat Ali − *Department of Agricultural Engineering, Khwaja Fareed University of Engineering & Information Technology, Rahim Yar Khan 64200, Pakistan;* orcid.org/0000-0002-5965-7352

Muhammad Akram − *Department of Botany, The Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan*

Baber Ali − *Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan;* orcid.org/0000-0003-1553-2248

Faisal Mahmood − *Department of Botany, The Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan*

Umbreen Shahzad − *Department of Horticulture, College of Agriculture, University of Layyah, Layyah 31200, Pakistan*

Mumtaz Hussain − *Department of Anatomy and Histology, Faculty of Veterinary Sciences, The Islamia University of Bahawalpur Pakistan, Bahawalpur 63100, Pakistan*

Mohamed Farouk Elsadek − *Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh 11362, Saudi Arabia*

Abd El-Zaher M. A. Mustafa − *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11362, Saudi Arabia*

Ijaz Ahmad − *Faculty of Agricultural Sciences and Department of Agronomy, Bahauddin Zakariya University, Multan 60800, Pakistan*

Reham M. Mostafa − *Department of Botany and Microbiology, Faculty of Science, Benha University, Benha 13518, Egypt*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c03238

Author Contributions

Conceptualization: M.S.H, N.N; H.A; Data curation: B.A; R.I; M.F.E; S.E; A.E.M.A.M; R.M.M; Formal analysis: B.A; R.I; M.F.E; S.E; A.E.M.A.M; R.M.M; Funding acquisition: M.F.E; S.E; A.E.M.A.M; Software:, Investigation, M.S.H, N.N; Methodology, M.S.H, N.N; H.A, B.A (Basharat Ali), M.A; Software, F.M, U.S, I.A, M.H; Writing − original draft: M.S.H, N.N; H.A, F.M, U.S, I.A, M.H; Writing − review & editing: M.S.H, N.N; H.A, B.A; R.I; M.F.E; S.E; A.E.M.A.M; R.M.M, M.S.H, N.N; H.A, B.A (Basharat Ali), M.A; Final Approval: All authors approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Researchers supporting project number (RSPD2023R941) King Saud University, Riyadh, Saudi Arabia.

REFERENCES

(1) Amna; Ali, B.; Azeem, M. A.; Qayyum, A.; Mustafa, G.; Ahmad, M. A.; Javed, M. T.; Chaudhary, H. J. Bio-Fabricated Silver Nanoparticles: A Sustainable Approach for Augmentation of Plant Growth and Pathogen Control. *Sustainable Agriculture Reviews*; Springer, 2021; pp 345−371.

(2) Saini, A.; Manuja, S.; Kumar, S.; Hafeez, A.; Ali, B.; Poczai, P. Impact of Cultivation Practices and Varieties on Productivity, Profitability, and Nutrient Uptake of Rice (Oryza Sativa L.) and Wheat (Triticum aestivum L.) Cropping System in India. *Agriculture* **2022**, *12*, 1678.

(3) Popp, J.; Pető, K.; Nagy, J. Pesticide productivity and food security. A review. *Agron. Sustain. Dev.* **2013**, *33*, 243−255.

(4) Tahir, O.; Bangash, S. A. K.; Ibrahim, M.; Shahab, S.; Khattak, S. H.; Ud Din, I.; Khan, M. N.; Hafeez, A.; Wahab, S.; Ali, B.; Makki, R. M.; Harakeh, S. Evaluation of Agronomic Performance and Genetic Diversity Analysis Using Simple Sequence Repeats Markers in Selected Wheat Lines. *Sustainability* **2023**, *15*, 293.

(5) Ali, H.; Tahir, M.; Nadeem, M. A. Determining Critical Period of Weed Competition in Wheat under Different Tillage Systems. *Pak. J. Life Soc. Sci.* **2014**, *12*, 74−79.

(6) Babar, M. A.; Khan, A.; Azam, S.; Ikramullah; Arif, M.; Hussain, S. Weeds in Wheat Crop: Weed Whole Plants Nutrient Concentration and Uptake under Fertilizer Application and Irrigation Frequencies. *Pure Appl. Biol.* **2019**, *8*, 1724−1735.

(7) Saeed, S.; Ullah, A.; Ullah, S.; Noor, J.; Ali, B.; Khan, M. N.; Hashem, M.; Mostafa, Y. S.; Alamri, S. Validating the Impact of Water Potential and Temperature on Seed Germination of Wheat (Triticum Aestivum L.) via Hydrothermal Time Model. *Life* **2022**, *12*, 983.

(8) Rafiq, M.; Saqib, M.; Jawad, H.; Javed, T.; Hussain, S.; Arif, M.; Ali, B.; Bazmi, M. S. A.; Abbas, G.; Aziz, M.; et al. Improving Quantitative and Qualitative Characteristics of Wheat (<i>Triticum aestivum</i> L.) through Nitrogen Application under Semiarid Conditions. *Phyton* **2023**, *92*, 1001−1017.

(9) Al-Huqail, A. A.; Saleem, M. H.; Ali, B.; Azeem, M.; Mumtaz, S.; Yasin, G.; Marc, R. A.; Ali, S. Efficacy of Priming Wheat (Triticum Aestivum) Seeds with a Benzothiazine Derivative to Improve Drought Stress Tolerance. *Funct. Plant Biol.* **2023**, DOI: 10.1071/fp22140.

(10) Asad, M.; Khan, R. R.; Aljuboory, A. B.; Rashid, M. H. U.; Kumar, U.; Haq, I. U.; Hafeez, A.; Noureldeen, A.; Alharbi, K. Toxic and Antifeedant Effects of Different Pesticidal Plant Extracts against Beet. *Phyton* **2023**, *92*, 1161.

(11) Marwat, S. K.; Usman, K.; Khan, N.; Khan, M. U.; Khan, E. A.; Khan, M. A.; ur Rehman, A. Weeds of Wheat Crop and Their Control Strategies in Dera Ismail Khan District; Khyber Pakhtun Khwa, Pakistan. *Am. J. Plant Sci.* **2013**, *4*, 66.

(12) Vikkey, H. A.; Fidel, D.; Pazou Elisabeth, Y.; Hilaire, H.; Hervé, L.; Badirou, A.; Alain, K.; Parfait, H.; Fabien, G.; Benjamin, F. Risk Factors of Pesticide Poisoning and Pesticide Users' Cholinesterase Levels in Cotton Production Areas: Glazoué and Savè Townships, in Central Republic of Benin. *Environ. Health Insights* **2017**, *11*, 117863021770465.
(13) Rao, A. N.; Chauhan, B. S. *Weeds and Weed Management in India—A Review*, 2015.
(14) Anwar, T.; Qureshi, H.; Perveen, N.; Mahmood, S.; Zulqurnain Haider, M.; Mumtaz, S.; Nawaz, H.; Khan, S. A.; Hafeez, A.; Imran Tipu, M.; Abd El-Rahim, I. H. A.; Alhag, S. K.; Ahmed, A. E. Herbicidal Effectiveness of Wild Poisonous Plant Rhazya Stricta Using Different Media by the Sandwich Method. *Pak. J. Bot.* **2023**, *55*, 2.
(15) Alam, J. N.; Amiri, H.; Javadi, M.; Gohlami, M. Efficiency of Different Herbicides Application for Weed Control in Wheat under Rain Fed Conditions in Iran. *Int. J. Agric. Crop Sci.* **2013**, *5*, 445−449.
(16) Ali, M.; Sabir, S.; Mohy-ud-din, Q.; Ali, M. A. Efficacy and economics of different herbicides against narrow leaved weeds in wheat. *Int. J. Agric. Biol.* **2004**, *6*, 647.
(17) Song, N. H.; Yin, X. L.; Chen, G. F.; Yang, H. Biological responses of wheat (Triticum aestivum) plants to the herbicide chlorotoluron in soils. *Chemosphere* **2007**, *68*, 1779−1787.
(18) Yin, X. L.; Jiang, L.; Song, N. H.; Yang, H. Toxic reactivity of wheat (Triticum aestivum) plants to herbicide isoproturon. *J. Agric. Food Chem.* **2008**, *56*, 4825−4831.
(19) Hassan, M. S.; Naz, N.; Ali, H.; Ali, B.; Akram, M.; Iqbal, R.; Ajmal, S.; Ali, B.; Ercisli, S.; Golokhvast, K. S.; et al. Ultra-Responses of Asphodelus Tenuifolius L.(Wild Onion) and Convolvulus Arvensis L.(Field Bindweed) against Shoot Extract of Trianthema Portulacastrum L.(Horse Purslane). *Plants* **2023**, *12*, 458.
(20) Jabeen, S.; Ali, M. F.; Mohi ud Din, A.; Javed, T.; Mohammed, N. S.; Chaudhari, S. K.; Javed, M. A.; Ali, B.; Zhang, L.; Rahimi, M. Phytochemical Screening and Allelopathic Potential of Phytoextracts of Three Invasive Grass Species. *Sci. Rep.* **2023**, *13*, 8080.
(21) Oracz, K.; Bouteau, H. E.; Farrant, J. M.; Cooper, K.; Belghazi, M.; Job, C.; Job, D.; Corbineau, F.; Bailly, C. ROS Production and Protein Oxidation as a Novel Mechanism for Seed Dormancy Alleviation. *Plant J.* **2007**, *50*, 452−465.
(22) Basu, S.; Rao, Y. V. Environmental Effects and Management Strategies of the Herbicides. *Int. J. Bio-Resour. Stress Manage.* **2020**, *11*, 518−535.
(23) Dahiya, S.; Kumar, S.; Khedwal, R. S.; Jakhar, S. R. Allelopathy for Sustainable Weed Management. *J. Pharmacogn. Phytochem.* **2017**, *6*, 832−837.
(24) Nasira, J.; Moinuddin, A.; Shaukat, S. S. Allelopathic Effects of Weeds on Wheat (Triticum Aestivum L.) Germination and Growth. *Pak. J. Bot.* **2013**, *45*, 807−811.
(25) Muhammad, Z.; Majeed, A. Allelopathic Effects of Aqueous Extracts of Sunflower on Wheat (Triticum Aestivum L.) and Maize (Zea Mays L.). *Pak. J. Bot.* **2014**, *46*, 1715−1718.
(26) Hamidi, D.; Ghadiri, H. Winter Wheat Cultivars Responses to Allelopathic Potential of Wild Barley (Hordeum Spontaneum Koch) Extracts. *J. Plant Breed. Crop Sci.* **2011**, *3*, 391−395.
(27) Al Sherif, E. A.; Gharieb, H. R. Allelochemical Effect of Trianthema Portulacastrum L. on Amaranthus Viridis L. Supports the Ecological Importance of Allelopathy. *Afr. J. Agric. Res.* **2011**, *6*, 6690−6697.
(28) Hassan, M. S.; Naz, N.; Ali, H. Evaluation of the Allelopathic Potential of Trianthema Portulacastrum L. on Convolvulus Arvensis L. *Biochem. Syst. Ecol.* **2022**, *104*, 104491.
(29) Richards, L. A. *Diagnosis and Improvement of Saline and Alkali Soils*; LWW, 1954; Vol. *78*.
(30) Arnon, D. I. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. *Plant Physiol.* **1949**, *24*, 1−15.
(31) Ali, B.; Hafeez, A.; Afridi, M. S.; Javed, M. A.; Sumaira; Suleman, F.; Nadeem, M.; Ali, S.; Alwahibi, M. S.; Elshikh, M. S.; Marc, R. A.; Ercisli, S.; Darwish, D. B. E. Bacterial-Mediated Salinity Stress Tolerance in Maize (Zea Mays L.): A Fortunate Way toward Sustainable Agriculture. *ACS Omega* **2023**, *8*, 20471−20487.
(32) Chance, B.; Maehly, A. C. [136] Assay of Catalases and Peroxidases. *Methods in Enzymology*; Academic Press, 1955.
(33) Kacar, B. *Practice Guide of Plant Nutrition*; Ankara Univ. Publ. Agric. Fac., 1984; Vol. *900*.
(34) Hamilton, P. B.; Van Slyke, D. D.; Lemish, S. The Gasometric Determination of Free Amino Acids in Blood Filtrates by the Ninhydrin-Carbon Dioxide Method. *J. Biol. Chem.* **1943**, *150*, 231−250.
(35) Petrova, S. T.; Valcheva, E. G.; Velcheva, I. G. A Case Study of Allelopathic Effect on Weeds in Wheat. *Ecol. Balk.* **2015**, *7*, 121.
(36) Kandhro, M. N.; Tunio, S. D.; Rajpar, I.; Chachar, Q. D.; Gandahi, A. W. Allelopathic Impact of Sorghum and Sunflower on Germination and Seedling Growth of Summer Broadleaf Weeds. *Pakistan J. Agric. Agric. Eng. Vet. Sci.* **2015**, *31*, 229−239.
(37) Asghar, M.; Tanveer, A.; Nadeem, M. A.; Ali, H. H. Comparative Allelopathic Potential of Two Aizoaceae Weeds against Germination of Different Crops. *Pakistan J. Weed Sci. Res.* **2013**, *19*, 377.
(38) Khaliq, A.; Matloob, A.; Khan, M. B.; Tanveer, A. Differential Suppression of Rice Weeds by Allelopathic Plant Aqueous Extracts. *Planta Daninha* **2013**, *31*, 21−28.
(39) Natarajan, A.; Elavazhagan, P.; Prabhakaran, J. Allelopathic Potential of Billy Goat Weed Ageratum Conyzoides L. and Cleome Viscosa L. on Germination and Growth of Sesamum Indicum L. *Int. J. Curr. Biotechnol.* **2014**, *2*, 21−24.
(40) Ghimire, B. K.; Hwang, M. H.; Sacks, E. J.; Yu, C. Y.; Kim, S. H.; Chung, I. M. Screening of Allelochemicals in Miscanthus Sacchariflorus Extracts and Assessment of Their Effects on Germination and Seedling Growth of Common Weeds. *Plants* **2020**, *9*, 1313.
(41) Jafariehyazdi, E.; Javidfar, F. Comparison of Allelopathic Effects of Some Brassica Species in Two Growth Stages on Germination and Growth of Sunflower. *Plant, Soil Environ.* **2011**, *57*, 52−56.
(42) Naeem, M.; Mahmood, A.; Ihsan, M. Z.; Daur, I.; Hussain, S.; Aslam, Z.; Zamanan, S. A. Trianthema Portulacastrum and Cyperus Rotundus Interference in Maize and Application of Allelopathic Crop Extracts for Their Effective Management. *Planta Daninha* **2016**, *34*, 209−218.
(43) Zohaib, A.; Abbas, T.; Tabassum, T. Weeds Cause Losses in Field Crops through Allelopathy. *Not. Sci. Biol.* **2016**, *8*, 47−56.
(44) Ramgunde, V.; Chaturvedi, A. Allelopathic Effect of Some Plants on Morphological Attributes of Invasive Alien Weed: Malachra Capitata (L.) L. *GSC Biol. Pharm. Sci.* **2019**, *6*, 108−114.
(45) Mahakhode, R. H.; Chaudhari, R. R.; Somkuwar, S. R. Influence of Glyphosate on Seed Germination and Seedling Growth of Psoralea Corylifolia L. *BIOINFOLET-A Q. J. Life Sci.* **2012**, *9*, 500−504.
(46) Akram, M.; Naz, N.; Ali, H. Anatomical and Physiological Systematics of Capparis Decidua (Forsskal.) Edgew from Different Habitats of Cholistan Desert, Pakistan. *Biochem. Syst. Ecol.* **2022**, *105*, 104539.
(47) Naz, N.; Batool, R.; Fatima, S.; Hameed, M.; Ashraf, M.; Ahmad, F.; Ahmad, M. S. A. Adaptive Components of Tolerance to Salinity in a Saline Desert Grass Lasiurus Scindicus Henrard. *Ecol. Res.* **2015**, *30*, 429−438.
(48) Al-maskri, A.; Hameed, M.; Ashraf, M.; Khan, M. M.; Fatima, S.; Nawaz, T.; Batool, R. Structural Features of Some Wheat (Triticum Spp.) Landraces/Cultivars under Drought and Salt Stress. *Arid Land Res. Manage.* **2014**, *28*, 355−370.
(49) Lei, Z. Y.; Han, J. M.; Yi, X. P.; Zhang, W. F.; Zhang, Y. L. Coordinated Variation between Veins and Stomata in Cotton and Its Relationship with Water-Use Efficiency under Drought Stress. *Photosynthetica* **2018**, *56*, 1326−1335.
(50) Zainab, N.; Amna; Khan, A. A.; Azeem, M. A.; Ali, B.; Wang, T.; Shi, F.; Alghanem, S. M.; Hussain Munis, M. F.; Hashem, M.; Alamri, S.; Abdel Latef, A. A. H.; Ali, O. M.; Soliman, M. H.; Chaudhary, H. J. Pgpr-Mediated Plant Growth Attributes and Metal

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX

Extraction Ability of Sesbania Sesban l. In Industrially Contaminated Soils. *Agronomy* **2021**, *11*, 1820.
(51) Mehmood, S.; Khatoon, Z.; Amna; Ahmad, I.; Muneer, M. A.; Kamran, M. A.; Ali, J.; Ali, B.; Chaudhary, H. J.; Munis, M. F. H. Bacillus Sp. PM31 Harboring Various Plant Growth-Promoting Activities Regulates Fusarium Dry Rot and Wilt Tolerance in Potato. *Arch. Agron. Soil Sci.* **2021**, *69*, 197−211.
(52) El-Badri, A. M. A.; Batool, M.; Mohamed, I. A. A.; Khatab, A.; Sherif, A.; Wang, Z.; Salah, A.; Nishawy, E.; Ayaad, M.; Kuai, J.; et al. Modulation of Salinity Impact on Early Seedling Stage via Nano-Priming Application of Zinc Oxide on Rapeseed (Brassica Napus L.). *Plant Physiol. Biochem.* **2021**, *166*, 376−392.
(53) Rasool, A.; Ghani, A.; Nawaz, R.; Ahmad, S.; Shahzad, K.; Rebi, A.; Ali, B.; Zhou, J.; Ahmad, M. I.; Tahir, M. F.; et al. Effects of Poultry Manure on the Growth, Physiology, Yield, and Yield-Related Traits of Maize Varieties. *ACS Omega* **2023**, *8*, 25766−25779.
(54) Ali, J.; Jan, I.; Ullah, H.; Fahad, S.; Saud, S.; Adnan, M.; Ali, B.; Liu, K.; Harrison, M. T.; Hassan, S.; et al. Biochemical Response of Okra (Abelmoschus Esculentus L.) to Selenium (Se) under Drought Stress. *Sustainability* **2023**, *15*, 5694.
(55) Ameen, F.; Mumtaz, S.; Ali, B.; Hussain, I.; Hafeez, A.; Gul, A.; Elsharkawy, M. M.; Hashim, T. A.; Yasin, G.; Khan, M. N.; et al. The Impact of Cu-Polluted and Organic Soil on the Fibrous Plant; Insights into Plant Growth Promotion, Antioxidant Defences System, and Oxidative Stress. *Funct. Plant Biol.* **2023**, DOI: 10.1071/fp23027.
(56) Alshegaihi, R. M.; Mfarrej, M. F. B.; Saleem, M. H.; Parveen, A.; Ahmad, K. S.; Ali, B.; Abeed, A. H. A.; Alshehri, D.; Alghamdi, S. A.; Alghanem, S. M. S.; Lone, J. A.; Soliman, T. M. A.; Soudy, F. A. Effective Citric Acid and EDTA Treatments in Cadmium Stress Tolerance in Pepper (Capsicum Annuum L.) Seedlings by Regulating Specific Gene Expression. *South Afr. J. Bot.* **2023**, *159*, 367−380.
(57) Shahzadi, E.; Nawaz, M.; Iqbal, N.; Ali, B.; Adnan, M.; Saleem, M. H.; Okla, M. K.; Abbas, Z. K.; Al-Qahtani, S. M.; Al-Harbi, N. A.; et al. Silicic and Ascorbic Acid Induced Modulations in Photosynthetic, Mineral Uptake, and Yield Attributes of Mung Bean (Vigna Radiata L. Wilczek) under Ozone Stress. *ACS Omega* **2023**, *8*, 13971−13981.
(58) Nankishore, A.; Farrell, A. D. The Response of Contrasting Tomato Genotypes to Combined Heat and Drought Stress. *J. Plant Physiol.* **2016**, *202*, 75−82.
(59) Rugare, J. T.; Pieterse, P. J.; Mabasa, S. Evaluation of the Potential of Jack Bean [Canavalia Ensiformis (L.) DC.] and Velvet Bean [Mucuna Pruriens (L.) DC.] Aqueous Extracts as Post-Emergence Bio-Herbicides for Weed Control in Maize (Zea Mays L.). *Asian J. Agric. Rural Dev.* **2020**, *10*, 420−439.
(60) Pompeiano, A.; Landi, M.; Meloni, G.; Vita, F.; Guglielminetti, L.; Guidi, L. Allocation Pattern, Ion Partitioning, and Chlorophyll a Fluorescence in Arundo Donax L. in Responses to Salinity Stress. *Plant Biosyst.* **2017**, *151*, 613−622.
(61) El-Shora, H. M.; El-Gawad, A.; Ahmed, M. Evaluation of Allelopathic Potential of Rumex Dentatus Root Extract and Allelochemicals on Cicer Arietinum. *J. Stress Physiol. Biochem.* **2014**, *10*, 167−180.
(62) Salam, A.; Afridi, M. S.; Javed, M. A.; Saleem, A.; Hafeez, A.; Khan, A. R.; Zeeshan, M.; Ali, B.; Azhar, W.; Sumaira; Ulhassan, Z.; Gan, Y. Nano-Priming against Abiotic Stress: A Way Forward towards Sustainable Agriculture. *Sustainability* **2022**, *14*, 14880.
(63) Asma; Hussain, I.; Ashraf, M. Y.; Saleem, M. H.; Ashraf, M. A.; Ali, B.; Shereen, A.; Farid, G.; Ali, M.; Shirazi, M. U.; Saleem, A.; et al. Alleviating Effects of Salicylic Acid Spray on Stage-Based Growth and Antioxidative Defense System in Two Drought-Stressed Rice (Oryza Sativa L.) Cultivars. Turkish. *J. Agric. For.* **2023**, *47*, 79−99.
(64) Azeem, M.; Pirjan, K.; Qasim, M.; Mahmood, A.; Javed, T.; Muhammad, H.; Yang, S.; Dong, R.; Ali, B.; Rahimi, M. Salinity Stress Improves Antioxidant Potential by Modulating Physio-Biochemical Responses in Moringa Oleifera Lam. *Sci. Rep.* **2023**, *13*, 2895.
(65) Abeed, A. H. A.; Saleem, M. H.; Asghar, M. A.; Mumtaz, S.; Ameer, A.; Ali, B.; Alwahibi, M. S.; Elshikh, M. S.; Ercisli, S.; Elsharkawy, M. M.; et al. Ameliorative Effects of Exogenous Potassium Nitrate on Antioxidant Defense System and Mineral Nutrient Uptake in Radish (Raphanus Sativus L.) under Salinity Stress. *ACS Omega* **2023**, *8*, 22575−22588.
(66) Kakar, H. A.; Ullah, S.; Shah, W.; Ali, B.; Satti, S. Z.; Ullah, R.; Muhammad, Z.; Eldin, S. M.; Ali, I.; Alwahibi, M. S.; Elshikh, M. S.; Ercisli, S. Seed Priming Modulates Physiological and Agronomic Attributes of Maize (Zea Mays L.) under Induced Polyethylene Glycol Osmotic Stress. *ACS Omega* **2023**, *8*, 22788−22808.
(67) Akram, N. A.; Iqbal, M.; Muhammad, A.; Ashraf, M.; Al-Qurainy, F.; Shafiq, S. Aminolevulinic Acid and Nitric Oxide Regulate Oxidative Defense and Secondary Metabolisms in Canola (Brassica Napus L.) under Drought Stress. *Protoplasma* **2018**, *255*, 163−174.
(68) Lukatkin, A. S.; Gar'kova, A. N.; Bochkarjova, A. S.; Nushtaeva, O. V.; Teixeira da Silva, J. A. Treatment with the Herbicide TOPIK Induces Oxidative Stress in Cereal Leaves. *Pestic. Biochem. Physiol.* **2013**, *105*, 44−49.
(69) Ullah, S.; Khan, M.; Khan, N.; Ali, U.; Ali, B.; Iqbal, R.; Z Gaafar, A. R.; Almunqedhi, B.; Razak, S. A.; Kaplan, A.; Ercisli, S.; et al. Efficacy of Naphthyl Acetic Acid Foliar Spray in Moderating Drought Effects on the Morphological and Physiological Traits of Maize Plants (*Zea mays* L.). *ACS Omega* **2023**, *8*, 20488−20504.
(70) Saleem, A.; Zulfiqar, A.; Ali, B.; Naseeb, M. A.; Almasaudi, A. S.; Harakeh, S. Iron Sulfate (FeSO4) Improved Physiological Attributes and Antioxidant Capacity by Reducing Oxidative Stress of Oryza Sativa L. Cultivars in Alkaline Soil. *Sustainability* **2022**, *14*, 16845.
(71) Faryal, S.; Ullah, R.; Khan, M. N.; Ali, B.; Hafeez, A.; Jaremko, M.; Qureshi, K. A. Thiourea-Capped Nanoapatites Amplify Osmotic Stress Tolerance in Zea Mays L. by Conserving Photosynthetic Pigments, Osmolytes Biosynthesis and Antioxidant Biosystems. *Molecules* **2022**, *27*, 5744.
(72) Sidhu, G. P. S.; Singh, H. P.; Batish, D. R.; Kohli, R. K. Alterations in Photosynthetic Pigments, Protein, and Carbohydrate Metabolism in a Wild Plant Coronopus Didymus L.(Brassicaceae) under Lead Stress. *Acta Physiol. Plant.* **2017**, *39*, 176.
(73) Maqbool, N.; Wahid, A.; Farooq, M.; Cheema, Z. A.; Siddique, K. H. M. Allelopathy and Abiotic Stress Interaction in Crop Plants. *Allelopathy: Current Trends and Future Applications*; Springer, 2013; pp 451−468.

https://doi.org/10.1021/acsomega.3c03238
ACS Omega XXXX, XXX, XXX−XXX