BIOASSAY OF MORPHO-ANATOMICAL RESPONSES OF *CHENOPODIUM ALBUM* L. UNDER *TRIANTHEMA PORTULACASTRUM* L. WATER EXTRACT

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***Abstract*:**Weeds are the main problems in agriculture production because weeds are silent burglars for nutrients, moisture, space, and sunlight in the major crops. They also produce allelochemicals that restrain the growth of crop plants. *Chenopodium album* is one of the 12 most booming colonizing weeds. Early emergence, rapid growth, long-term seed viability, and high seed production give a competitive advantage to this weed over crop plants. To control this weed, chemical herbicides are usually applied but this technique faces many challenges such as the development of herbicide resistance in weeds along with damaging influence on the environment and human health. An answer to this crisis could be found in the development of biological weed control methods to combat and minimize the negative effects of synthetic herbicides. In conflict with these challenges, allelopathy is the most effective method to eradicate this weed. To evaluate the efficacy of shoot water extract of *Trianthema portulacastrum* was used along with the synthetic herbicide. The experiment was conducted by growing the *C. album* in pots. The shoot extract showed negative effects on morphological and anatomical parameters. The weed plant showed specific modifications in root and stem anatomy (such as a decrease in the cortical thickness, metaxylem cell area, and vascular bundle area), and leaf anatomy (increase in vascular bundle area, metaxylem cell area, and lamina thickness with a decrease in stomatal density) as adaptive characteristics to cope with biotic stress. It was concluded that shoot extract treatment posed a negative effect on weed growth by modifications in anatomical parameters due to malfunctioning in metabolic activities, therefore shoot extract is helpful for crop plants by destroying weeds through an environmentally friendly and cheap weed control technique.

***Index-terms*:** Allelopathic water extract; Anatomy; *Chenopodium*; Herbicide; Root length; Shoot length; *Trianthema*.

I. INTRODUCTION

Weeds are one of the mainly solemn problems in agriculture production because weeds and crop plants' requirements are identical. So there is eternal competition between them. They are silent burglars for nutrients, moisture, space, and sunlight in the major crops (Dash *et al*., 2020). Weeds require nitrogen and sunlight for the early stage of growth and soil moisture during fruit and seed formation, which eventually reduces the growth and yield of the major crops. They also produce allelochemicals that restrain the growth of crop plants (Ikram *et al*., 2020). *Chenopodium album* (Lamb's quarters or goose foot) exists all over the world and it is one of the 12 most booming colonizing weeds. It thrives on all types of soils. Early emergence, rapid growth, long-term seed viability (several decades), and high seed production (200-75000 per plant) give a competitive advantage to this weed over crop plants (Bhatia *et al.*, 2020). Weed control is very important in every crop production system because they pose a serious limitation to global agricultural output by reducing two-fifth of productivity. For weed management, chemical herbicides are usually applied because they are

relatively effective to provide instant weed control and involve less labor as compared to mechanical control. But chemical weed control faces many challenges such as the development of herbicide resistance in weeds along with damaging influence on the atmosphere and disparaging impacts on humans (Petrova *et al*., 2015). An answer to this crisis could be found in the development of biological weed control methods to combat and minimize the negative effects of synthetic herbicides (Balasko *et al.*, 2020).

Among integrated weed control, allelopathy is a foremost technique of environmental protection currently with increased weed management and energy economy. These natural allelochemicals have stumpy environmental hazards as compared to the conventional used chemical herbicides and a lower risk of resistance remains in the environment. It is, therefore, the motive to develop natural biochemicals which are an alternative to synthetic herbicides (El-Abbassi *et al*., 2017). The allelopathic perspective of a plant species is accredited to its secondary metabolites which may be mostly inhibitory in action. These allelochemicals have great potential for the development of bio-pesticides because they are free from tribulations coupled with pesticides. In conflict with the challenges of ecological contamination and opposition to herbicides, allelopathy is the most effective method to eradicate weeds (Sindhu *et al.*, 2018). Therefore to achieve sustainable weed management, allelopathy can be proposed as one of the probable substitutes and effective tools in weed controlling (Ikram *et al*., 2020).

T. portulacastrum is an annual herbaceous weed growing in summer crops. It contains secondary metabolites such as alkaloids, flavonoids, and tannins (Kondalkar *et al.*, 2019). These allelochemicals may encourage growth at lower levels and repress the growth at higher levels of neighboring plants. Allelopathy is now a forthcoming area that engrosses controlling weeds without any future disadvantages. There is no information accessible on the allelopathic effects of *T. portulacastrum* on *C. album* in comparison to synthetic weedicide. Consequently, the current research was carried out to investigate the substitute eco-friendly techniques for controlling the *C. album* because many studies have shown cases of resistance of this weed against synthetic herbicides like metribuzin, linuron, and atrazine (Petrova *et al*., 2015). In comparison with synthetic herbicides, the crude weed extract did not exterminate this weed completely, but the concentrated extract successfully eradicated the weed and overcame weed crop competition and thus increasing crop yield.

II. MATERIALS AND METHODS

2.1 Preparation of allelopathic water extract

T. portulacastrum plants were collected from the field during the summer season. Whole plants were rinsed thoroughly with distilled water to get rid of the dust on the leaves and soil from the roots. Shoots from a collected plant of *T. portulacastrum* were separated and dried under shade. Then, the dried samples were chopped in a grinder into a fine powder. The water extract was ready by soaking 10 grams of powder of shoot powder in 100 mL water (10% w/v) for 24 hours. After filtration of the extract with Whatman No.1 filter paper, the final concentrations of 30%, 60%, and 100% of the shoot extracts were prepared with dilution with distilled water (Hassan *et al.*, 2022).

2.2 Treatment groups applied to C. album

T1 = Control treatment

T2= 30% Allelopathic aqueous extract of shoot

T3= 60% Allelopathic aqueous extract of shoot

T4= 100% Allelopathic aqueous extract of shoot

T5= Herbicide treatment

Figure 1. *Trianthema portulacastrum* L. (A weed used as shoot allelopathic extract).

2.3 C. album seeds collection

The seeds of *C. album* were taken at the maturity stage from the wheat fields in April 2017. Seeds were stored at room temperature in paper envelopes.

2.4 Experiment conduction

A pot trial was performed at the Islamia University of Bahawalpur, Punjab, Pakistan (71.7647° E and 29.3783° N). After imbibition of seeds in cold water for 48 hours, 15 selected seeds of *C. album* were sown in each pot having 4 kg soil in November 2017, and then the experiment was repeated in 2018. The pots were regularly irrigated with tap water to keep up the moisture in the soil. After the emergence had been completed, thinning was done and ten healthy seedlings were left in each pot. After two months, in each pot 10 mL shoot aqueous extracts (30%, 60%, and 100%) of *T. portulacastrum* were applied to the soil of T2, T3 and T4 labeled pots respectively. While tape water and synthetic herbicide (Metafin Super 28.6% WDG) were applied to T1 and T5 respectively. The data was collected at the vegetative stage after the application of all treatments. Plant samples were collected after the ten days of treatment applied to study the morphology and anatomy.

2.5 Morphological Characteristics

Following morphological characteristics like root dry weight (g/plant), shoot dry weight (g/plant), shoot length (cm), shoot fresh weight (g/plant), leaf area (cm^2), root length (cm), a number of leaves ($plant^{-1}$), and root fresh weight (g/plant) were studied.

2.6 Anatomical Characteristics

For compound microscopic examination, *C. album* root, stem, and leaf specimens were prepared by killing and fixing in F.A.A. (10 ml formalin, 35 ml ethyl alcohol 95%, and 5ml glacial acetic acid). After free-hand sectioning, the standard double-stained technique was used for sections. Photomicrographs of prepared slides were recorded with a digital camera equipped with a Nikon stereo-microscope (Nikon 104, Japan). Various anatomical characteristics of the stem, root, and leaf (epidermal and cortical thickness, root area, vascular bundle area, phloem thickness, metaxylem cell area, and abaxial stomatal density) were calculated.

2.7 STATISTICAL ANALYSIS

The trial was accomplished in a completely randomized design with three replicates. ANOVA and LSD tests according to Steel and Torrie method (1960) were implemented on data by using the software "Statistix version 8.1". Pearson's correlation was calculated by "Microsoft Excel" and "Statistix version 8.1" between morphology and anatomy (root, stem, and leaf) of *C. album.*

III. RESULTS

3.1 MORPHOLOGICAL CHARACTERISTICS

C. album depicted a consistent and gradual decline in all morphological characteristics to an increasing level of shoot extract of *T. portulacastrum* at the vegetative stage. Diluted shoot extract caused a slight reduction in root dry weight, shoot dry weight, leaf area, shoot fresh weight, number of leaves, shoot length, and root fresh weight while more concentrated extract caused a greater decline in above mentioned morphological parameters. Analogous results were observed with herbicide treatment (Table 1).

Table 1. ANOVA for the influence of shoot extract of *T. portulacastrum* and herbicide on various morphological parameters of *C. album* at the vegetative stage.

Treatment levels	T1	T2	T3	T4	T5	LSD	CV	GM	F value
Morphological parameters									
Root length (cm)	18	16	13	8	2	0.79	3.8	11.4	667**
Shoot length (cm)	19	15	15	8	4	0.7	3.17	12.2	734**
Root fresh weight (g)	1.9	1.2	0.9	0.6	0.5	0.74	40.18	1.02	5.66*
Shoot fresh weight (g)	9	7.5	6.8	3.9	2.5	1.14	10.57	5.94	54.3**
Root dry weight (g)	0.5	0.42	0.21	0.21	0.11	0.1	19.01	0.29	26.2**
Shoot dry weight (g)	2.6	2.1	1.8	0.9	0.3	0.6	21.54	1.54	23.5**
Number of leaves ($plant^{-1}$)	17	13	13	8	3	2.7	13.73	10.8	39.8**
Leaf area (cm^2)	4.5	3.4	2.6	2	1.6	0.42	8.24	2.82	74.6**

T1= distilled water; T2= 30% extract; T3= 60% extract; T4= 100% extract; T5= herbicide treatment. LSD = Least significant difference; CV = Coefficient variation; GM = Grand mean; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; NS = Non-significant.

3.2 ANATOMICAL CHARACTERISTICS

Various anatomical modifications of the root, stem, and leaf of *the C. album* were calculated. This reduction pattern was also calculated at all shoot extract levels in all root parameters (epidermal thickness, cortical thickness, phloem thickness, metaxylem cell area, root area, and vascular bundle area). 30% shoot extract caused minimum reduction while a maximum decline was calculated at 100% shoot extract. A similar reduction trend was recorded in all parameters of root at herbicide treatment (Table 2 and Figure 2).

In the stem, most of the parameters decreased such as cortical cell area, phloem thickness, metaxylem cell area, pith area, vascular bundle area, and stem area. While endodermal thickness and epidermal thickness increased in the current study (Table 2). But in the case of the leaf, phloem thickness, upper epidermal thickness, and abaxial stomatal density declined in all shoot extract treatments, and more reduction was recorded at more concentrated levels. But metaxylem cell area increased at all shoot extract treatments except at 30% shoot extract level. (Table 2 and Figure 2).

Table 2. ANOVA for the influence of shoot extract of *T. portulacastrum* and herbicide on various anatomical parameters of *C. album* at the vegetative stage.

Treatment levels	T1	T2	T3	T4	T5	LSD	CV	GM	F value
Root anatomical parameters									
Epidermal thickness (µm)	0.50 a	0.42 ab	0.35 bc	0.29 c	0.46 a	0.1	14	0.4	6.73**
Cortical thickness (µm)	5.0 a	3.8 b	3.5b	3.1 b	2.0 c	0.98	15.5	3.48	12.2 **
Phloem thickness (µm)	2.0 a	1.9 ab	1.7 ab	1.3 ab	1.0 b	0.95	33.4	1.58	1.91 ^{NS}
Metaxylem cell area (µm^2)	1.30 a	1.10 ab	0.80 bc	0.70 c	0.50 c	0.35	22.1	0.88	8.05**
Vascular bundle area (µm^2)	21 a	18 b	16 b	12 c	5d	2.04	7.79	14.4	91.3**
Root area (µm^2)	25 a	16 b	11 c	9 d	6 e	1.31	5.39	13.4	318 **
Stem anatomical parameters									
Epidermal thickness (µm)	0.35 b	0.42a	0.35 b	0.32 b	0.42 a	0.06	9.54	0.37	4.93 *
Cortical thickness (µm)	5.0a	4.80 a	4.3 ab	3.5 b	2.0 c	1.1	15.4	3.92	12.3 **
Endodermal thickness (µm)	0.50 c	0.70 b	0.85 a	0.66 b	0.54 c	0.11	9.71	0.65	14.5 **
Phloem thickness (µm)	1.4 a	1.2a	1.1 ab	0.8 b	0.4 c	0.32	18.2	0.98	14.3 **
Metaxylem cell area (µm^2)	1.0 a	0.5 b	0.4 bc	0.3 bc	0.31 c	0.17	18.5	0.51	26.0 **
Pith area (µm^2)	8:00 AM	7:00 AM	5 b	5 b	4.6 b	1.5	13.9	5.92	9.82 **
Vascular bundle area (µm^2)	38a	37 a	31 b	31 b	29c	1.9	3.14	33.2	44.6 **
Stem area (µm^2)	61 a	57 ab	49 bc	49 bc	45 c	10.8	11.3	52.2	3.68*
Leaf anatomical parameters									
Upper epidermal thickness (µm)	1.0 a	0.7 ab	0.4 bc	0.3 c	0.1 c	0.34	38	0.5	10.4 **
Vascular bundle area (µm^2)	2.6 b	2.7 b	2.9 ab	3.1 a	3.2 a	0.35	6.72	2.9	5.13*
Metaxylem cell area (µm^2)	0.3 a	0.27a	0.32 a	0.33 a	0.35a	0.1	17	0.31	0.98 ^{NS}
Phloem thickness (µm)	1.2 a	1.2 a	0.9 b	0.79 b	0.72 b	0.23	13.2	0.97	9.32 **
Lamina thickness (µm)	7.0 ab	7.2 ab	7.5 a	7.5 a	6.3 b	1.1	8.77	7.1	1.89 ^{NS}

Abaxial stomatal density	24 a	21 b	17c	17 c	20 b	1.3	3.57	19.8	52.2 **

T1= distilled water; T2= 30% extract; T3= 60% extract; T4= 100% extract; T5= herbicide treatment; LSD = Least significant difference; CV = Coefficient variation; GM = Grand mean; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; NS = Non-significant.

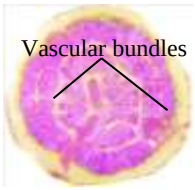
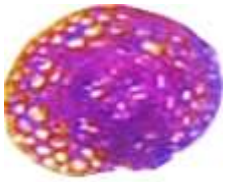
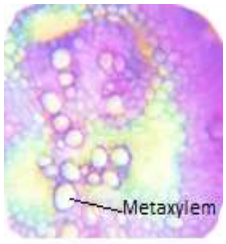
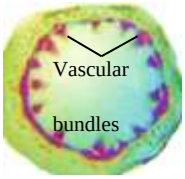
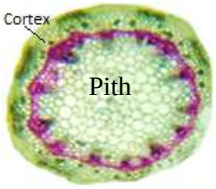
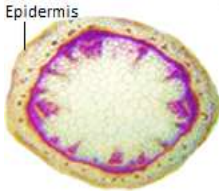
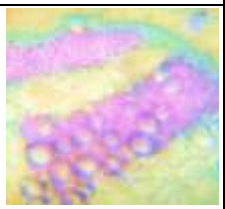
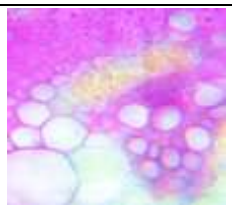
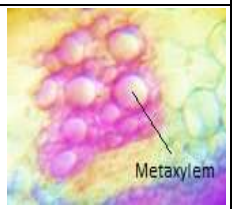
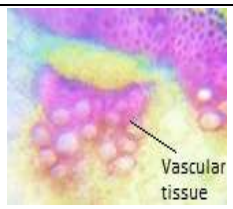
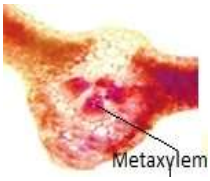
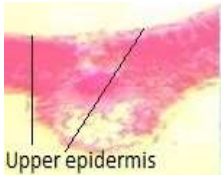
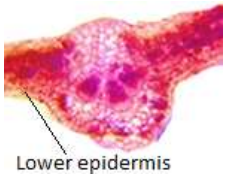
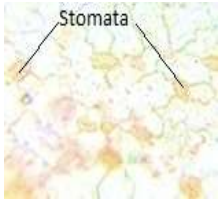
	T1 (Distilled water)	T2 (30% extract)	T3 (60% extract)	T4 (100% extract)	T5 (Herbicide treatment)
Root					
Root tissues					
Stem					
Stem tissues					
Leaf					
Leaf tissues					

Figure 2. Root, stem, and leaf anatomy of *C. album* with applying different levels of shoot extract of *T. portulacastrum* and herbicide.

3.3 *Correlations between morphological and root anatomical characteristics*

In *C. album* root length showed positive significant correlation with shoot length, root dry weight, shoot dry weight, number of leaves, leaf area, shoot fresh weight, cortical thickness, phloem thickness, metaxylem cell area, root fresh weight, vascular bundle area and root area; shoot length with root fresh weight, shoot dry weight, shoot fresh weight, leaf area, cortical thickness, phloem thickness, root dry weight, metaxylem cell area, number of leaves, vascular bundle area, and root area; root fresh weight with shoot fresh weight, number of leaves, root dry weight, leaf area, cortical thickness, phloem thickness, shoot dry weight, metaxylem cell area, vascular bundle area, and root area; shoot fresh weight with shoot dry weight, leaf area, vascular bundle area, cortical thickness, root dry weight, phloem thickness, number of leaves, metaxylem cell area, and root area; root dry weight with number of leaves, vascular bundle area, leaf area, cortical thickness, phloem thickness, metaxylem cell area, shoot dry weight, and root area; shoot dry weight with cortical thickness, number of leaves, leaf area, vascular bundle area, phloem thickness, metaxylem cell area, and root area; number of leaves with leaf area, phloem thickness, metaxylem cell area, cortical thickness, vascular bundle area, and root area; leaf area with cortical thickness, phloem thickness, metaxylem cell area, root area, and vascular bundle area; vascular bundle area, cortical thickness with metaxylem cell area, and root area; phloem thickness with metaxylem cell area, and root area; metaxylem cell are and vascular bundle area with root area; and vascular bundle area with root area. While epidermal thickness showed a negative correlation with shoot length, root fresh weight, shoot fresh weight, root length, shoot dry weight, number of leaves, vascular bundle area, root dry weight, cortical thickness, leaf area, phloem thickness, metaxylem cell area, and root area (Table 3).

Table 3. Correlation between morphology with root anatomy of *C. album*.

	RL	SL	RFW	SFW	RDW	SDW	NL	LA	ET	CT	PT	MXCA	VBA	RA
RL														
SL	0.98													
RFW	0.88	0.89												
SFW	0.98	0.99	0.92											
RDW	0.91	0.86	0.94	0.90										
SDW	0.99	0.99	0.92	1.00	0.91									
NL	0.98	0.99	0.89	0.98	0.86	0.99								
LA	0.92	0.92	0.99	0.95	0.97	0.95	0.92							
ET	0.22	0.28	0.59	0.35	0.47	0.31	0.22	0.53						
CT	0.95	0.95	0.95	0.95	0.92	0.96	0.97	0.96	0.30					
PT	1.00	0.98	0.88	0.99	0.90	0.99	0.98	0.92	0.27	0.93				
MXCA	0.95	0.92	0.96	0.95	0.99	0.96	0.92	0.99	0.44	0.96	0.95			
VBA	0.99	0.98	0.87	0.98	0.89	0.99	0.99	0.92	0.17	0.97	0.98	0.94		
RA	0.88	0.88	0.99	0.91	0.96	0.91	0.89	0.99	0.55	0.96	0.87	0.97	0.88	

Significant Level	Positive Correlation	Negative Correlation
0.05		
0.01		
0.001		

RL=Root length; RDW=Root dry weight; RFW=Root fresh weight; SL=Shoot length; SFW=Shoot fresh weight SDW=Shoot dry weight; LA=Leaf area; NL= number of leaves; ET=Epidermal thickness; CT=Cortical thickness; PT=Phloem thickness; MXCA=Metaxylem cell area; VBA=Vascular bundle area; RA=Root area.

3.4 *Correlations between morphological and stem anatomical characteristics*

In *C. album* root length showed positive significant correlation with shoot length, root dry weight, number of leaves, shoot fresh weight, leaf area, root fresh weight, cortical thickness, vascular bundle area, shoot dry weight, phloem thickness, pith area, and stem area; shoot length with shoot fresh weight, number of leaves, shoot dry weight, leaf area, cortical thickness, root dry weight, phloem thickness, root fresh weight, and stem area; root fresh weight with number of leaves, root dry weight, leaf area, phloem thickness, metaxylem cell area, shoot fresh weight, vascular bundle area, pith area, shoot dry weight, and stem area; shoot fresh weight showed positive correlation with cortical thickness, number of leaves, leaf area, phloem thickness, root dry weight, pith area, shoot dry weight, vascular bundle area, and stem area; root dry weight with number of leaves, shoot dry weight, cortical thickness, vascular bundle area phloem thickness, metaxylem cell area, pith area, leaf area, and stem area; shoot dry weight with number of leaves, leaf area, cortical thickness, metaxylem cell area, phloem thickness, pith area, vascular bundle area, and stem area; number of leaves with cortical thickness, leaf area, phloem thickness, and stem area; leaf area with metaxylem cell area, phloem thickness, pith area, vascular bundle area, cortical thickness, and stem area; cortical thickness with phloem thickness, stem area and vascular bundle area; phloem thickness with stem area and vascular bundle area; metaxylem cell area with pith area and stem area; pith area showed positive correlation with vascular bundle area and stem area; and vascular bundle area with stem area.

While epidermal thickness showed a non-significant correlation with shoot length, root dry weight, number of leaves, shoot fresh weight, root fresh weight, leaf area, cortical thickness, root length, endodermal thickness, phloem thickness, metaxylem cell area, shoot dry weight, pith area, vascular area, and stem area endodermal thickness with root length, number of leaves, root dry weight, shoot dry weight, root fresh weight, shoot length, leaf area, shoot fresh weight, and epidermal thickness; metaxylem cell area with number of leaves, cortical thickness, shoot fresh weight, shoot length, phloem thickness shoot dry weight, root length, and pith area; pith area with shoot length, number of leaves, epidermal thickness, cortical thickness, and phloem thickness; vascular bundle area with shoot length, number of leaves, epidermal thickness, and metaxylem cell area; and stem area with endodermal thickness and epidermal thickness (Table 4).

Table 4. Correlation between morphology with stem anatomy of C. album.

	RL	SL	RFW	SFW	RDW	SDW	NL	LA	ET	CT	EnT	PT	MXCA	PiA	VBA	SA
RL																
SL	0.98															
RFW	0.88	0.89														
SFW	0.98	0.99	0.92													
RDW	0.91	0.86	0.94	0.9												
SDW	0.99	0.99	0.92	0.99	0.91											
NL	0.98	0.99	0.89	0.98	0.86	0.99										
LA	0.92	0.92	0.99	0.95	0.97	0.95	0.92									

ET	-0.2	-0.23	-0.11	-0.14	-0.04	-0.18	-0.31	-0.1								
CT	0.99	0.96	0.81	0.96	0.87	0.97	0.97	0.87	-0.27							
EnT	0.16	0.16	-0.3	0.1	-0.24	0.09	0.15	-0.22	-0.18	0.24						
PT	0.99	0.99	0.88	0.98	0.89	0.99	0.99	0.92	-0.28	0.99	0.16					
MXCA	0.74	0.77	0.96	0.8	0.87	0.8	0.78	0.93	-0.21	0.68	-0.48	0.76				
PiA	0.85	0.81	0.95	0.86	0.99	0.87	0.81	0.97	0.04	0.79	-0.37	0.83	0.9			
VBA	0.89	0.83	0.91	0.88	0.99	0.89	0.83	0.95	0.05	0.9	-0.24	0.86	0.83	0.98		
SA	0.9	0.86	0.95	0.9	0.99	0.91	0.87	0.97	-0.06	0.86	-0.26	0.89	0.89	0.99	0.99	1

RL=Root length; RFW=Root fresh weight; RDW=Root dry weight; SL=Shoot length; SFW=Shoot fresh weight SDW=Shoot dry weight; NL= number of leaves; LA=Leaf area; ET=Epidermal thickness; CT=Cortical thickness; EnT=Endodermal thickness; PT=Phloem thickness; MXCA=Metaxylem cell area; PiA=Pith area; VBA=Vascular bundle area; SA=Stem area.

3.5 *Correlations between morphological and leaf anatomical characteristics*

In *C. album* root length showed positive significant correlation with shoot fresh weight, root dry weight, number of leaves, root fresh weight, leaf area, shoot dry weight, upper epidermal thickness, shoot length, and phloem thickness; shoot length with root dry weight, shoot dry weight, leaf area, shoot fresh weight, upper epidermal thickness, number of leaves, root fresh weight, and phloem thickness; root fresh weight with shoot fresh weight, number of leaves, root dry weight, upper epidermal thickness, shoot dry weight, leaf area, and phloem thickness; shoot fresh weight with number of leaves, leaf area, upper epidermal thickness, shoot dry weight, and root dry weight, phloem thickness; root dry weight with shoot dry weight, leaf area, upper epidermal thickness, number of leaves, and phloem thickness; shoot dry weight with leaf area, number of leaves, upper epidermal thickness, and phloem thickness; upper epidermal thickness, number of leaves with leaf area, and phloem thickness; leaf area with upper epidermal thickness and phloem thickness; upper epidermal area with phloem thickness; and upper epidermal area with metaxylem cell area. While vascular bundle area showed a negative significant correlation with shoot dry weight, shoot length, shoot fresh weight, number of leaves, root fresh weight, leaf area, root length, upper epidermal thickness, and root dry weight; metaxylem cell area with root length and root dry weight; and phloem thickness showed with vascular bundle area and metaxylem cell area. But metaxylem cell area showed a negative non-significant correlation with shoot length, root fresh weight, shoot fresh weight, number of leaves, shoot dry weight, leaf area, and upper epidermal thickness; and lamina thickness and abaxial stomatal density showed a non-significant correlation with all parameters (Table 5).

Table 5. Correlation between morphology with leaf anatomy of *C. album.*

	RL	SL	RFW	SFW	RDW	SDW	NL	LA	UET	VBA	MXCA	PT	LT	AbSD
RL														
SL	0.98													
RFW	0.88	0.89												
SFW	0.98	0.99	0.92											
RDW	0.91	0.86	0.94	0.9										
SDW	0.99	0.99	0.92	0.99	0.91									

NL	0.98	0.99	0.89	0.98	0.86	0.99								
LA	0.92	0.92	0.99	0.95	0.97	0.95	0.92							
UET	0.93	0.91	0.98	0.94	0.99	0.95	0.92	0.99						
VBA	-0.97	-0.95	-0.94	-0.98	-0.96	-0.98	-0.94	-0.97	-0.97					
MXCA	-0.86	-0.76	-0.68	-0.81	-0.85	-0.82	-0.75	-0.76	-0.79	0.87				
PT	0.94	0.9	0.9	0.94	0.96	0.94	0.88	0.94	0.95	-0.99	-0.93			
LT	0.52	0.47	0.12	0.4	0.22	0.44	0.52	0.2	0.24	-0.3	-0.38	0.25		
AbSD	0.48	0.48	0.8	0.55	0.75	0.54	0.45	0.76	0.74	-0.66	-0.46	0.68	-0.46	

RL=Root length; RDW=Root dry weight; RFW=Root fresh weight; SL=Shoot length; SDW=Shoot dry weight; NL= number of leaves; SFW=Shoot fresh weight; LA=Leaf area; UET=Upper epidermal thickness; VBA=Vascular bundle area; MXCA= Metaxylem cell area; PT= Phloem thickness; LT= Lamina thickness; AbSD =Abaxial stomatal density.

IV. DISCUSSION

The most efficient technique to destroy weeds in agriculture is to employ herbicides. They are non-degradable and pollute water and soil. While allelochemicals are free from all these hazards (Zeng, 2014). Therefore, these are very imperative for new and eco-friendly pesticide detection (Scavo and Mauromicale, 2021).

The current study revealed the decline in root and shoot length of *C. album* with the allelopathic treatment of *T. portulacastrum* which was supported by Shafique *et al*. (2011), who reported the suppression of the shoot length and root length of *Avena sativa* with the aqueous extract of *C. murale*. Our results are analogous to the work of Sutradhar *et al*. (2018), who illustrated that the more root length was found in the lowest concentration while the less root length was recorded in the highest concentration of extract solution. Similarly, *T. portulacastrum* L. and S. *portulacastrum* L. extracts significantly repressed the root and shoot length in tested species (Asghar *et al*., 2013). Safdar *et al.* (2019) illustrated the reduction in plant height of wheat infested with *C. arvensis*. The length of the root and shoot of *Convolvulus arvensis* was significantly decreased by water extracts of *T. portulacastrum* (Hassan *et al*., 2022). Various phenolic acids (ferulic acid, caffeic acid, M- coumaric acid, P- coumaric acid, syringic acid, vanillic acid; and gallic acid, caffeic acid, 4-Hydroxy-3-Methoxybenzoic acid, P- coumaric acid, syringic acid) were found in the *T. portulacastrum* (Al-Sherif and Gharieb, 2011). They mainly contributed to the inhibition of metabolic processes by reducing the activity of GA and IAA. This dive in hormones causes a reduction of mitosis and the growth of a plant (Schaller *et al*., 2014). Plenty of allelochemicals such as chlorogenic acid, ferulic acid, p- coumaric acid, caffeic acid, 4-hydroxy-3-methoxy benzoic acid, gallic acid, and vanillic acid caused interruption of plant growth (Asghar *et al*., 2013). Allelochemicals dwindled the elongation, expansion, and division of cells due to impaired metabolic activities (Algandaby and Salama, 2018.).

The chemicals like phenolics, terpenoids, and alkaloids, and their derivatives are potential inhibitors of fresh weight and dry weight (Natarajan *et al*., 2014). Shoot, root fresh and dry weight of *Triticum* diminished gradually as the concentration of powder was increased. A higher amount of allelopathic extract from three weeds (*Asphodelus,*

Euphorbia, and *Fumaria*) reduced or completely ceased the wheat growth (Nasira *et al*., 2013). Fresh weight and dry weight reduction in our study were also supported by Naeem *et al*. (2018), who described the decline of the dry weight of *C. album* and *Cronopus didymus* with the synthetic herbicide application. The decline in fresh and dry biomass of *C. album* may be coupled to decrease root and shoot length due to the existence of allelopathic compounds in the extract of *T. portulacastrum*. These compounds limit water and nutrient uptake by roots and lessen photosynthesis, poor stomatal regulations, and biomass accumulation (Akram *et al*., 2018).

A considerable reduction in the leaf area of soybean, sorghum, and groundnut was in response to allelopathic residues of *Cyperus rotundus* and *Commelina benghalensi.* This suppression outcome was increased with an enhancement in the level of weed residues (Jalageri *et al*., 2010). Abbas *et al*. (2018) confirmed by a bioassay study that *Malva parviflora* and *Chenopodium murale* aqueous water extracts posed a negative effect on the leaf area of weed plants. Drought stress caused a decline in the number of leaves in *Rosa damascena* (Hessini *et al*., 2022).

The root because of its direct connection with the soil is the first site that shows the response against any kind of stress. The epidermal thickness of the root in *Chenopodium album* reduced significantly almost at all levels of extracts as well as at herbicide treatment. Naz *et al*. (2013) reported a decline in root epidermal thickness of *Aeluropus logopoides* with an increase in abiotic stress. Similarly, cortical thickness also decreased more or less at all treatments. Similar work was reported by Hameed *et al.* (2020) in wheat lines by applying leaf extract of *Alstonia scholaris.* This reduction in the cortical region, resulting a decrease in the storage capacity of plants and raises the level of sensitivity, and may cause acute tissue damage in plants (Burgos *et al*., 2004). The decline in metaxylem cell area increases the efficacy of water and mineral translocation in plants. In the present work, a slight decrease in metaxylem cell area at all levels of shoot water extract was recorded. But a sharp reduction in herbicide treatment was noticed. Naz *et al*. (2016) proved that increase in the level of abiotic stress (salinity) caused a decrease in the area of metaxylem vessels in *Sporobolus ioclados*. Root area was significantly reduced in the present study. Hameed *et al.* (2020) described similar outcomes in root area in wheat lines with *Alstonia scholaris* allelochemicals extract of a leaf. It may be because of a decrease in cortical parenchyma, and in some cases due to pith parenchyma cells and vascular tissue by allelochemicals.

Epidermis thickness plays an important role in adjusting water level. An increase in epidermal thickness of the stem at most of the treatments of shoot water extract as well as at herbicide treatment was calculated. Sousa *et al*. (2021) reported similar results and said that with the treatment of Epibrassinolide (EBR) there was an increase in the epidermal thickness of plants to cope with water stress. The cortical cell area at all shoot extracts levels was decreased in the stem. Parallel findings were described by Akram *et al*. (2002) who described a reduction in the cortical cell area of wheat in saline conditions (abiotic stresses). Vascular bundle area reduction and metaxylem area increase are also reported by Wasim and Nargis (2020) at high stress in *Cenchrus ciliaris.* Herbicide application leads to anatomical modifications in plants such as the demolition of stem cortical and pith cells (Mahakhode and Somkuwar (2012). The stem area at herbicide treatment and the shoot water extract was decreased. In general, plants display a reduction in stem area when grown in saline conditions, as previously identified in *Lupinus termis* by Shaaban *et al*. (2022). When plant tissues are

exposed to stress conditions, they established cell size reduction and increased vascular tissue and cell wall thickness for their survival (Guerfel *et al*., 2009).

The upper epidermal thickness of the leaf decreased at all levels of shoot extracts and also at herbicide treatment. This decline in results was parallel with the outcomes of Akram *et al*. (2002), who stated a decline in the *Triticum aestivum* leaf epidermis growing under abiotic stress (salt stress). An increase in metaxylem cell area was recorded at higher concentrations of shoot extract levels. This increase in metaxylem area suggested stronger tolerance to high abiotic stresses, as water and mineral translocation may be easier and minimize the conduction resistance (Chen *et al*., 2021). An increase in lamina thickness is supported by Wasim and Nargis (2020) at high stress in the leaf of *Cenchrus ciliaris.* Stomatal density reduction is the major adaptation for the compensation of water loss. Stomatal density at the abaxial epidermis invariably declined except for 30% shoot extract and this character supports under stressful environmental conditions. Lesser stomatal density caused a reduction in transpiration rate, which helps plants to grow more successfully. Reduction in stomatal number may be more advantageous for the regulation of transpiration rate. A decline in stomatal density at herbicide treatment was parallel to the investigation of Semerdijieva *et al*. (2015), who reported a decline in the number of stomata with the application of herbicides. Hameed *et al.* (2020) also reported a reduction in abaxial stomatal density in wheat lines at higher levels of leaf extract of *Alstonia scholaris.*

V. CONCLUSION

The current study revealed that *T. portulacastrum* allelopathic water extract of shoot caused an allelopathic impact on the morphological and anatomical parameters of the *C. album* due to the presence of various secondary metabolites. Various modifications in anatomical parameters were recorded at shoot water extract as well as at herbicide treatment. However, the effect of a higher concentration of shoot water extract was similar to the commercially synthetic herbicides according to the results of measurements and analysis. Therefore, allelopathic potentials of shoot of *T. portulacastrum* may endorse them to control specific weeds, particularly in non-sequential crops by preparing them as natural herbicides.

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Conflict of interest

The authors declare no conflict of interest.

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