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Article

Dactyloctenium aegyptium (L.) Willd. (Poaceae) Differentially Responds to Pre- and Post-Emergence Herbicides through Micro-Structural Alterations

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Abstract: Herbicides are widely used to kill weeds and increase crop production all over the world. Nevertheless, some weeds show certain structural modifications in response to herbicide application that impart mostly partial or sometimes complete tolerance to these noxious plants. The present study was focused on morpho-anatomical modifications in the root, stem, and leaves of *Dactyloctenium aegyptium* (L.) Willd. treated with different herbicides and to examine whether it possesses tolerance against herbicides. Two pre- and four post-emergence herbicides were applied to *D. aegyptium* at the recommended dose in a randomized complete block design (RCBD). Pre-emergence herbicide Bromoxynil enhanced root growth (30%), leaves per plant (3%), and leaf fresh weight (17.2%). Increased stem epidermal thickness (100%) was the most notable feature among anatomical attributes. Post-emergence herbicides generally increased stem epidermal thickness 33–56%), leaf sheath thickness (5%), and root area in roots. Other modifications included increased sclerenchymatous thickness in the stem (133–255%), and epidermal thickness (100–200%) in the leaf blade. These characters assisted *D. aegyptium* to cope with herbicide toxicity. Collectively, pre-emergence herbicides more effectively controlled *D. aegyptium* compared with post-emergence herbicides.

Keywords: Egyptian crowfoot grass; pre-emergence; post-emergence; herbicide tolerance; anatomical modifications

1. Introduction

Conventional and mechanical techniques to eradicate weeds are too laborious. This has strongly encouraged modern-day farmers to use modern technologies for the elimination of noxious weeds from cultivated fields. The use of chemicals for the removal of unwanted plants is very economic and time saving [1] though it raises many ecological and human health concerns. Weed control depends on the emergence period of weeds, herbicide application time, mode of application (pre- or post-emergence), and type of crops [2].

Weeds have evolved certain avoidance, tolerance, and resistance mechanisms for their survival in response to the continuous application of herbicides. They have developed sunken stomata, thick waxy cuticle layers on the upper surface of the leaf, and enclosed growing points that prevent herbicide penetration inside the plant body. Herbicides disturb the functioning of a few key enzymes that catalyze the production of specific amino acids in plants. The survival of weeds is enhanced by overcoming phytochemical-induced toxicities [3].

Herbicides may be selective or non-selective and kill all plants. Though most of the commercial herbicides efficiently kill many weedy plants, some weeds might develop tolerance to these chemical herbicides. Some populations of weeds have the ability to tolerate herbicide toxicity due to the presence of tolerant alleles originating from random DNA mutations [4]. Tolerant alleles regulate several highly constitutive tolerance mechanisms that prevent herbicides from damaging key metabolic pathways. Several defense mechanisms prevent herbicides from reaching target proteins [4]. The important mechanisms are reduced cellular uptake or translocation within plants via vacuolar sequestration or detoxification that change the biochemical properties of herbicides through herbicide catabolism [5].

Some herbicides tolerate weeds that exhibit certain micro-structural adaptations such as dense cuticles that decrease herbicide penetration, a high proportion of storage parenchyma for sequestering herbicide away from the metabolically active sites, and a thicker epidermis to reduce uptake and alterations in the structure of target sites [6]. In some cases, weeds try to sequester chemical herbicides at the site of application through a reduction in phloem tissue for decreased translocation, while an expanded cortical region increases storage capacity at local sites where the herbicide is absorbed [7]. Opinions of the researchers on herbicide usage for weed control are divided. One group favors it while the other group is strongly against extensive herbicide usage [8]. However, the use of these weed control agents has certain advantages in reducing crop losses and in yield improvement, their residual effects are even of more concern than the benefits. The routine use of herbicides in significant quantities is associated with soil, air, and water pollution, and the poisoning, toxicity, disease, and death of organisms. This causes significant economic losses and raises ecological concerns [8]. Highly volatile herbicides drift away from the crops and cause environmental pollution. Some herbicides such as organophosphates and carbamates are nerve gases that affect the brain and nervous systems in animals. Another example is the use of fumigants such as methyl bromide that can damage tissues on contact. Even after harvest, weedicides have certain residual effects such as preventing decomposition by reducing mold and fungal activities [8].

Dactyloctenium aegyptium (Egyptian crowfoot grass) is a C4 perennial weed of the tropics, subtropics, and warm temperate regions [9] and mostly grows in moist soils [10]. In Pakistan, it is extensively distributed in Sindh, Punjab, Khyber Pakhtoonkhwa, and Kashmir. *Dactyloctenium aegyptium* grows well on a wide range of soils including alkaline and saline soil [11]. Although it is an important component of herbivores in natural ecosystems and for grazing cattle and small ruminants in fallow lands, it emerges as a noxious weed in agricultural field where it is the most difficult to control. Since many herbicide-tolerant weeds modify their structural traits for the survival against post- and pre-emergence herbicides, it was hypothesized that *D. aegyptium*, a herbicide-tolerant weed, should have developed certain micro-structural alterations to effectively curtail the damaging effects of the applied chemical herbicides. Based on this hypothesis, the research questions framed in this study include: (i) How does *D. aegyptium* respond to the recommended doses of tested herbicides at morphological and anatomical levels? (ii) What type of micro-structural and morphological adaptations helps curtail the damaging effects of these chemical herbicides? (iii) Are the induced micro-structural modifications herbicide specific or common to all herbicides? (iv) Can the tolerance mechanisms/alterations be classified by mode of application, i.e., pre- or post-emergence? (v) Do all applied herbicides treatments equally influence the observed plant growth traits or otherwise?

2. Materials and Methods

Anatomical features are more responsive to environmental conditions than morphological and physiological modifications, but unfortunately this is an ignored field. The anatomical response of Dactyloctenium aegyptium has never been evaluated for tolerance in response to different herbicides.

2.1. Experimental Layout

The structural defense of Egyptian crowfoot grass (*Dactyloctenium aegyptium*) against herbicides applied at the pre- and post-emergence stages was evaluated. The fields were irrigated once and ploughed before the sowing of weeds at Ayyub Agriculture Research Institute according to the layout plan as presented in Supplementary Figure S1. A mixture of weed seeds were sown in the first week of September 2018. The monocotyledonous weeds included grasses such as Bermuda grass (*Cynodon dactylon* (L.) Pers.), wild oats (*Avena fatua* L.), Egyptian crowfoot grass (*Dactyloctenium aegyptium* (L.) Willd.), and lesser-canary grass (*Phalaris minor* Retz.). Dicotyledonous species were lamb's-quarters (*Chenopodium album* L.), nettleleaf goosefoot (*C. murale* L.), bindweed (*Convolvulus arvensis* L.), sun spurge (*Euphorbia helioscopia* L.), and toothhead dock (*Rumex dentatus* L.). A combination of different pre- and post-emergence herbicides were applied to weeds. The survival rate of *D. aegyptium* was the maximum; therefore, it was selected for further studies. The experiment was laid out in a randomized complete block design (RCBD) with 3 replications.

2.2. Herbicide Application

Six post- and pre-emergence herbicides were selected for the experiment. The herbicides that are widely used in Pakistan for the eradication of weeds were selected. Pre–emergence herbicides were sprayed three days after sowing before the weeds' seeds emerged out of the soil. The pre-emergence herbicides used were (i) Acetamide @ 2250 mL/ha and (ii) Bromoxynil @ 2250 mL/ha. Post-emergence herbicides were sprayed two weeks after sowing when the seedlings were about 6 cm in height. The post–emergence herbicides included (i) Metolachlor-Atrazine @ 2000 mL/ha post-emergence, (ii) Methyl ester @ 50 g/ha, (iii) Mesotrione @ 2000 mL/ha, and (iv) Atrazine-Mesotrione-Halosulfuron methyl @ 2000 mL/ha. The generic names and the modes of action of the herbicides are presented in Table 1. Chemical names, modes of action of the herbicides, and percent mortality of *D. aegyptium* by herbicide application are presented in Table 1. Both groups of herbicides were sprayed between 10 and 11 a.m. in the morning when the dew was dry and air movement was minimal. Plastic sheets were installed around the fields to prevent the herbicides from drifting from one field to the other.

2.3. Herbicide Tolerance Criteria

After four weeks of herbicide spray, the survival of weeds was evaluated based on the percent mortality rate. The mortality rate of *Dactyloctenium aegyptium* ranged from 25 ± 2 to 41.7 ± 3% after the application of different herbicides. Therefore, it was selected for the detailed study of micro-structural features involved in its survival to a wide range of herbicides.

2.4. Collection of Plant Material and Growth Measurements

Six plants (n = 42) from each treatment and each replication were carefully uprooted by auger and placed on a metallic sieve plate (dia. 30 cm). The roots were then washed carefully to remove soil and dried on a blotting paper. Fresh weights of the root and shoot were taken immediately on a portable top-load balance. The plants were then wrapped carefully in a wet towel and placed in sealed plastic bags for transferring to the laboratory. Plant height, root length, and leaf sheath length were measured by a scale. Dry weights were measured after drying the plant in an oven at 60 °C for one week. The number of leaves per plant was counted and leaf area was measured by portable leaf area meter (LI-3000C, Lincoln, NE 68504, USA).

2.5. Anatomical Measurements

For anatomical characteristics, root, stem, and leaf samples of *D. aegyptium* were separated and immediately preserved in formalin aceto-alcohol solution (formalin 5%, acetic acid 10%, ethanol 50%, and distilled water 35%) for 48 h. It was subsequently transferred to acetic alcohol solution (acetic acid 25% and ethanol 75%) for long-term preservation. Permanent slides were prepared by free-hand sectioning. Thin sections were then selected and dehydrated in serial ethanol grades (30, 50, 70, 95, and 100% ethanol). Two different biological stains (safranin for lignified cell walls and fast green for primary wall) were then used for developing a contrast. After staining, the samples were cleared with xylene and mounted by Canada balsam on a glass slide. Measurement of different cells and tissues was taken by using an ocular micrometer pre-calibrated with a stage micrometer. Photographs were taken on a camera-equipped stereo-microscope (Saitama 354-0043, Meiji Techno, Japan).

2.6. Statistical Analysis

The first research question, i.e., whether the recommended doses of tested herbicides were effective in controlling *D. aegyptium* was evaluated by determining the growth and anatomical effects caused by herbicides. The data collected were subjected to one-way analysis of variance (ANOVA) by keeping the herbicides as main effects using CoStat statistical package (v 6.303) and the LSD values were used to test the significance of mean values for various plant parameters. Pearson's correlation matrixes were constructed in R (i386 4.0.5) to visualize the relationship between the groups of variables (morphology, root stem, leaf, and leaf blade anatomy) under collective herbicide application. The second question, i.e., what type of micro-structural and morphological adaptations play a role in curtailing the damaging effects of the chemical herbicides was addressed by determining the changes in cellular structures of various tissues, i.e., root, stem, leaf, and leaf sheath. A redundancy analysis (RDA) was then run to construct RDA triplots with the assumption that growth attributes (response variables as factor 1) were regulated by root, shoot, leaf, and leaf sheath anatomical attributes (dependent variables as factor 2) under the influence of herbicide spray (control variables as factor 3). The growth attributes were plotted separately with root, shoot, leaf, and leaf sheath anatomy in CanoDraw (v 4.14) software supplied with Canoco (v 4.5) in RDA triplots. The third research question whether micro-structural modifications were herbicide specific or were common to all herbicides was accessed by constructing heatmaps where different attributes (clustered in columns) were clustered in response to the herbicide application treatments (clustered in rows) using R (i386 4.0.5). For a better visualization, the heatmap graphs were broken into slices, both for plant attributes and herbicides, from a second branch using a customized R code. To evaluate the fourth research question, i.e., whether the tolerance mechanisms/alterations can be classified by mode of application, i.e., pre- or post-emergence, a combined PCA biplot was constructed in R (i386 4.0.5). Then two types of eclipses were drawn (i) for grouping of plant attributes to individual herbicide treatments and (ii) for grouping of plant attributes based on mode of application, i.e., pre-emergence and post-emergence. The fifth question, i.e., whether all applied herbicides treatments equally influence the observed plant growth traits was addressed by clustering herbicides based on all recorded growth and anatomical traits using NtSys-pc program 2.11X [12]. All six herbicide treatments were treated as influential fixed operational units (OUs) and the variable characters (morphological and anatomical attributes). Pairwise distances on "interval data" of all pairs of taxa were computed using Euclidean (EUCLID) distance coefficients with the average distance. The distance matrix was used for Unweighted Pair Group Method with Arithmetic mean (UPGMA) cluster analysis using the Sequential Agglomerative Hierarchical Non-overlapping (SAHN) method [13].

The distinct branches of the trees constructed were used to classify all six herbicide treatments into an identifiable cluster.

3. Results

3.1. Anatomical Measurements

3.1.1. Morphological Attributes

Herbicide treatments significantly reduced the plant height of *D. aegyptium*. Pre-emergence Acetamide was the most affective herbicide causing about a 57% decrease in plant height (Figure 1). Post-emergence herbicides such as Methyl ester, Mesotrione, and Atrazine-Mesotrione-Halosulfuron methyl decreased plant height from 30 to 40%. When compared with control plants, pre-emergence Acetamide caused a 30% decrease in root length, while post-emergence herbicides such as Metolachlor-Atrazine, Mesotrione, and Atrazine-Mesotrione-Halosulfuron methyl reduced root length by 10–20%. Root length increased in Bromoxynil-treated plants by 30%. Application of pre-emergence Acetamide resulted in a decrease of 35–40% in leaf area, whereas Methyl ester, Mesotrione, and Atrazine-Mesotrione-Halosulfuron methyl decreased this trait by up to 20–40% compared to the control. Pre-emergence Acetamide decreased the number of leaves per plant by 20–25%, while post-emergence Mesotrione and Atrazine-Mesotrione-Halosulfuron methyl-treated plants reduced the number of leaves by 15–20% compared to the control. Length of leaf sheath was not much affected by post- or pre-emergence herbicides. The most effective herbicide on root biomass was Acetamide, causing a 57% decline in root fresh weight over the control. Methyl ester, Mesotrione, and Atrazine-Mesotrione-Halosulfuron methyl, all post-emergence herbicides, decreased root fresh weight by 20–25%. Acetamide significantly reduced the stem fresh weight of the plant (about 55%). Stem fresh weight was reduced by 25–35% over the control when treated with post-emergence herbicides such as Atrazine-Mesotrione-Halosulfuron methyl and Mesotrione. Leaf fresh weight was decreased by 45% as compared to the control under the effect of the most effective pre-emergence Acetamide. Post-emergence herbicides, i.e., Mesotrione and Atrazine-Mesotrione-Halosulfuron methyl caused a 30% reduction in leaf fresh weight (Figure 1).

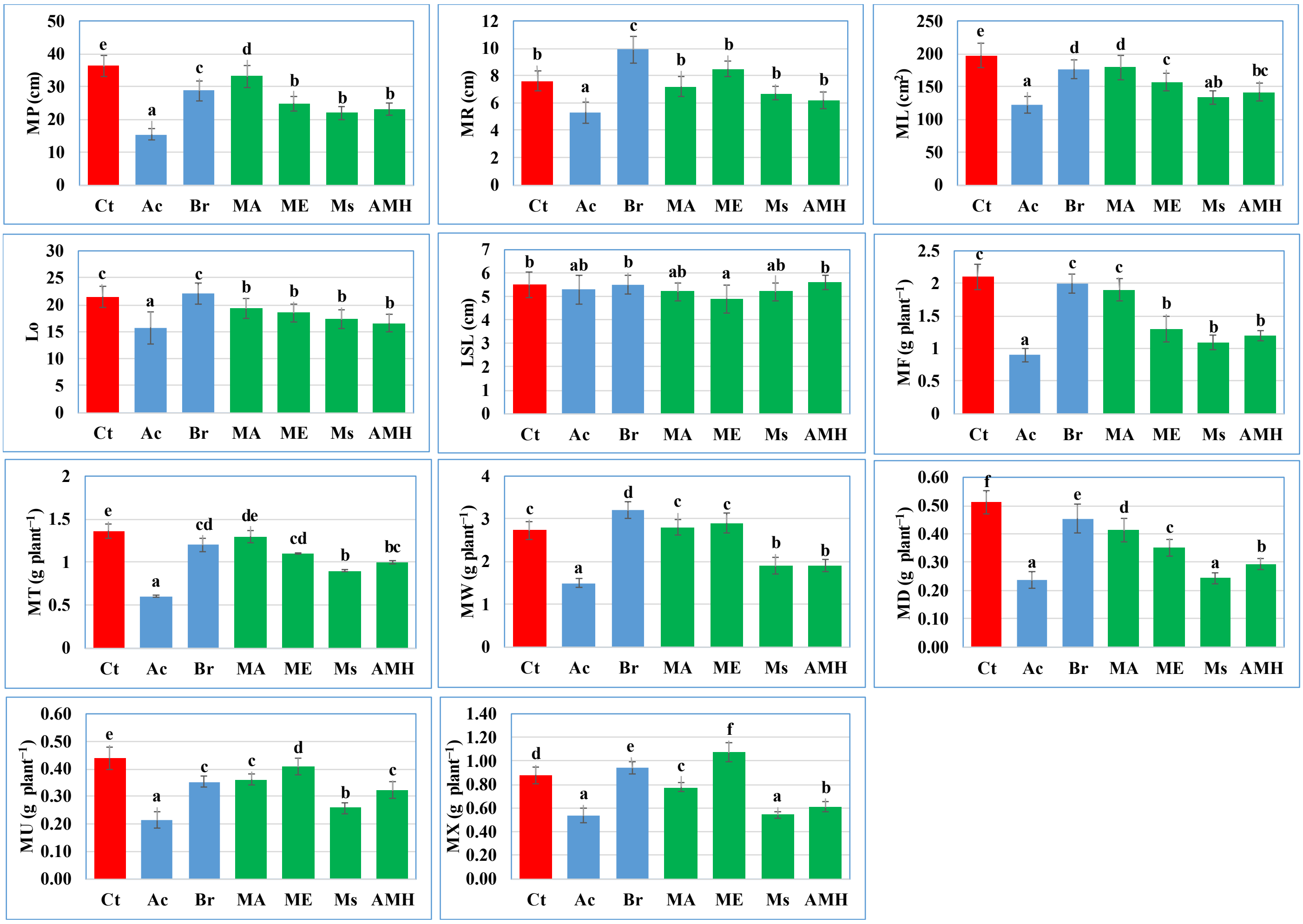


Figure 1. Morphological characteristics of *Dactyloctenium aegyptium* treated with pre- and post-emergence herbicides. Means sharing same lowercase letters on bars are statistically non−significant at $p \leq 0.05\%$). Red bar indicates control group, blue bars show pre-emergence and green bars are used for post-emergence herbicides. For abbreviations please see list at the end of manuscript.

3.1.2. Root Anatomy

Root radius was decreased by 55% when treated with pre-emergence Acetamide, while Bromoxynil reduced this parameter by 26% (Figure 2 and Supplementary Figure S2). Post-emergence herbicides decreased root radius from 4 to 17% as compared to the control. Epidermal thickness of *D. aegyptium* was decreased by 33% when treated with Acetamide, Metolachlor-Atrazine, and Mesotrione. Bromoxynil decreased epidermal thickness by 17% over the control while Methyl ester and Atrazine-Mesotrione-Halosulfuron methyl exhibited non-significant variation as compared to the untreated control. Three herbicides (Acetamide, Metolachlor-Atrazine, and Methyl ester) resulted in an increase in epidermal cell area over the untreated control, where the maximum increase over the control was observed in Methyl ester (13%). The most effective were the post-emergence Mesotrione and Atrazine-Mesotrione-Halosulfuron methyl causing a 24 and 28% decrease in epidermal cell area as compared to the control. Cortical region thickness was most severely affected by post-emergence Atrazine-Mesotrione-Halosulfuron methyl, where an 88% decrease was noted. Pre-emergence Bromoxynil decreased cortical thickness by 43%, whereas Acetamide, Metolachlor-Atrazine, and Mesotrione decreased this trait by 10–13% over the control. The most effective herbicides were pre-emergence. Bromoxynil and post-emergence

Mesotrione which decreased cortical cell area by 80–90% as compared to the untreated control.

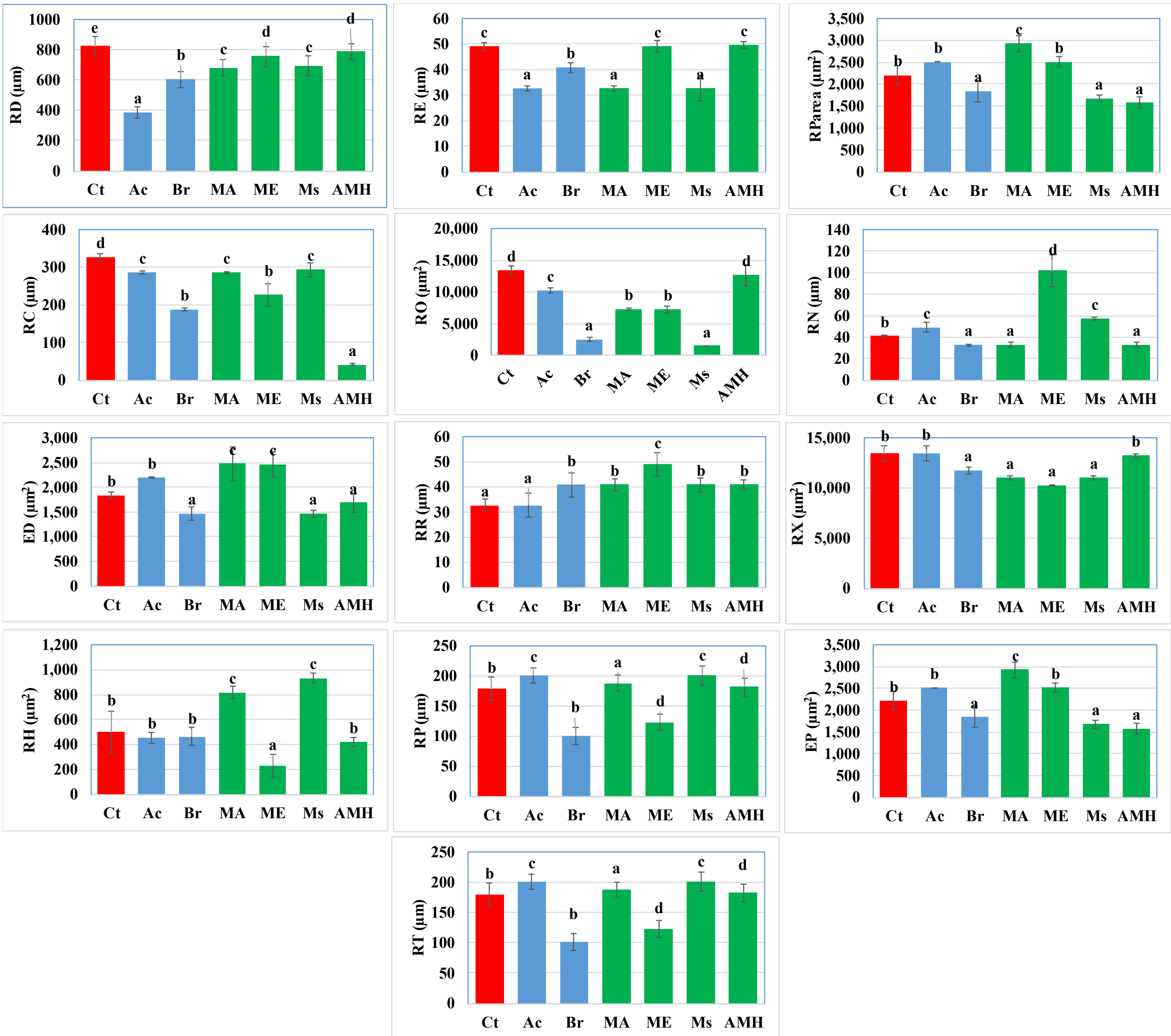


Figure 2. Root anatomical characteristics of *Dactyloctenium aegyptium* treated with pre- and post-emergence herbicides. Means sharing same lowercase letters on bars are statistically non−significant at $p \leq 0.05$%). Red bar indicates control group, blue bars show pre-emergence and green bars are used for post-emergence herbicides. For abbreviations please see list at the end of manuscript.

(Figure 2) Metolachlor-Atrazine, and Methyl ester decreased this attribute by 45%, whereas Atrazine-Mesotrione-Halosulfuron methyl did not alter the cortical cell area. Root endodermal thickness increased with three herbicides; the maximum increase was noted in plants treated with Methyl ester showing a 150% increase over the untreated control. This attribute was increased by 20% over the control when treated with Acetamide and 40% in Mesotrione-treated plants. Endodermal cell area was increased by 34–35% in Metolachlor-Atrazine- and Methyl ester-treated plants. Pre-emergence Acetamide showed a 20% increase in this parameter as compared to the control, while other herbicides depicted a significant decrease. Pericycle thickness was not affected by pre-emergence Acetamide, while it increased with all other herbicides (Supplementary Figure S2).

Application of post-emergence Methyl ester to *Dactyloctenium aegyptium* showed the maximum increase (50%) in pericycle thickness. Metaxylem area was decreased by 13–23% as compared to the control in four herbicides as compared to the untreated control. This trait was not affected by pre-emergence Acetamide and post-emergence Atrazine-Mesotrione-Halosulfuron methyl. Phloem area was significantly increased by two post-emergence herbicides. Mesotrione showed an increase of 87% and Metolachlor-Atrazine by 64% as compared with the untreated control. Methyl ester adversely affected the phloem area, showing a 55% decrease over the untreated control. Pith cell area was decreased in two herbicides, where a 44% decrease was noted in pre-emergence Bromoxynil-treated plants and 31% in post-emergence Methyl ester-treated plants. Pre-emergence Acetamide and Mesotrione resulted in an increase in this parameter as compared to the untreated control.

3.1.3. Stem Anatomy

Stem radius decreased significantly in all herbicide treatments and the maximum decrease was noted in Atrazine-Mesotrione-Halosulfuron methyl-treated plant where a 47% reduction was noted (Supplementary Figure S2 and Figure 3). Bromoxynil treatment showed a 7% decrease in stem radius, while other herbicides exhibited a 25–28% decrease over the control. Epidermal thickness increased in two treatments, pre-emergence Bromoxynil and post-emergence Mesotrione, where a 100% increase was noticed. Metolachlor-Atrazine and Atrazine-Mesotrione-Halosulfuron methyl (both post-emergence herbicides) treatment significantly decreased epidermal thickness by up to 80%. Epidermal cell area significantly increased in four treatments, and these all were post-emergence herbicides. The maximum increase (330%) was recorded in Methyl ester-treated plants. Mesotrione and Atrazine-Mesotrione-Halosulfuron methyl showed a 112% increase in this trait, while Metolachlor-Atrazine resulted in a 77% increase as compared to the control. Pre-emergence Acetamide and Bromoxynil, and post-emergence Mesotrione and Atrazine-Mesotrione-Halosulfuron methyl showed a significant increase in cortical cell area, where the maximum increase was found in Mesotrione-treated plants (56%). Post-emergence Metolachlor-Atrazine and Methyl ester did not affect the cortical cell area of *D. aegyptium* (Figure 3). Sclerenchymatous thickness increased in all herbicide treatments except Atrazine-Mesotrione-Halosulfuron methyl. The maximum increase (255%) was noted in Methyl ester-treated plants. Post-emergence Metolachlor-Atrazine increased this attribute by 233% and Mesotrione exhibited a 133% increase over control. Vascular bundle area decreasedwith pre-emergence herbicides Acetamide and Bromoxynil, resulting in a 21 and 32% reduction, respectively. This trait increased in all post-emergence herbicides except Methyl ester, and the maximum increase was seen in Metolachlor-Atrazine (21%). Metaxylem area significantly decreased in all herbicide treatments; the only exception was Methyl ester where no change was observed. The most effective herbicide was Mesotrione causing a 70% decrease in metaxylem area. Phloem area generally increased in herbicide-treated plants, while only Mesotrione showed a reduction (46%) in this attribute. The maximum increase was recorded in post-emergence Metolachlor-Atrazine (145%). Pre-emergence Acetamide, Bromoxynil, and post emergence Atrazine-Mesotrione-Halosulfuron methyl increased this parameter by 32–49% as compared to the control.

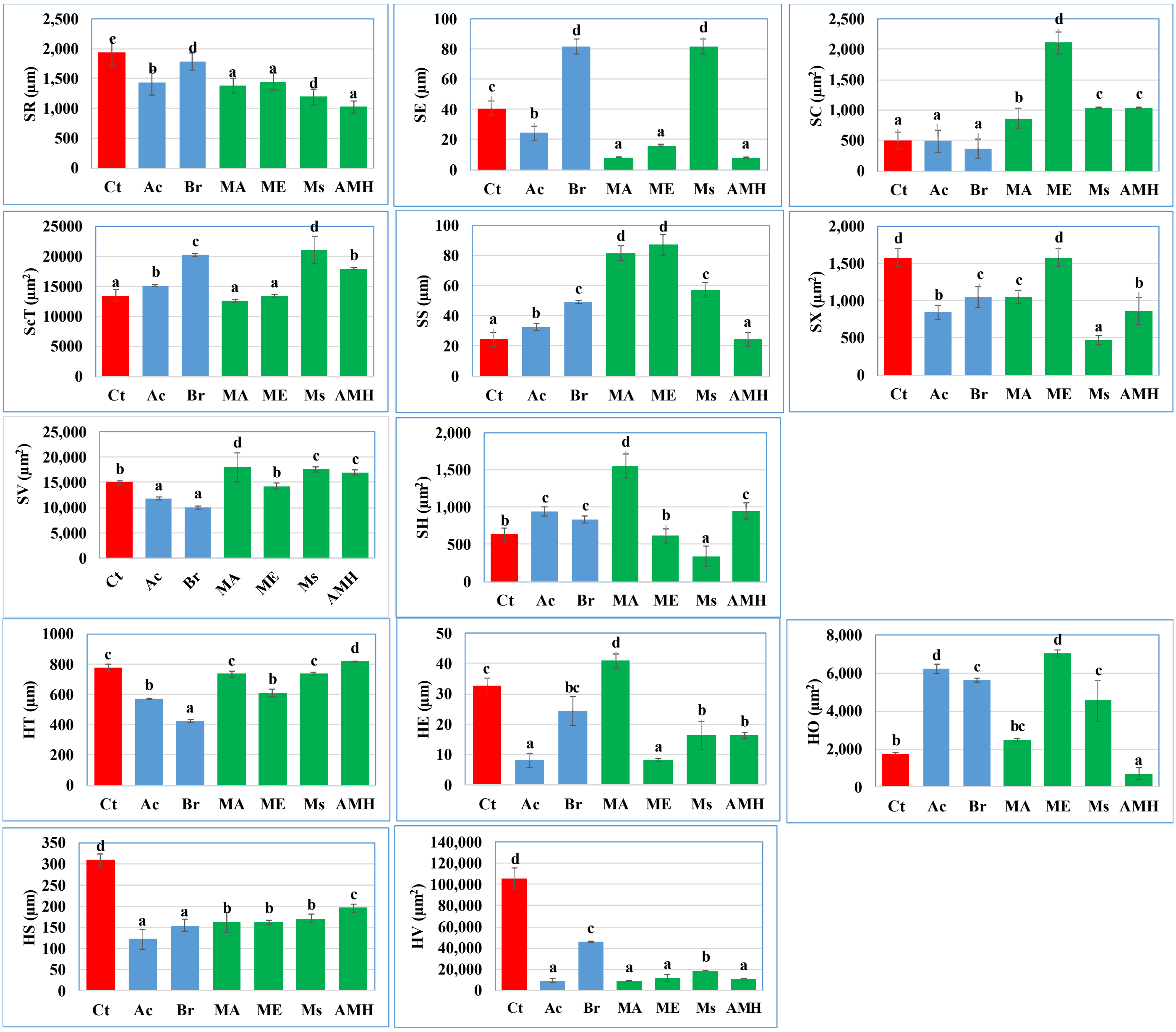


Figure 3. Stem and leaf sheath anatomical characteristics of *Dactyloctenium aegyptium* treated with pre- and post-emergence herbicides. Means sharing same lowercase letters on bars are statistically non−significant at $p \leq 0.05\%$) Red bar indicates control group, blue bars show pre-emergence and green bars are used for post-emergence herbicides. For abbreviations please see list at the end of manuscript.

3.1.4. Leaf Sheath Anatomy

Leaf sheath thickness significantly increased only in post-emergence herbicides Atrazine-Mesotrione-Halosulfuron methyl showing a 5.2% increase over control. In pre-emergence herbicides the most effective herbicide was Bromoxynil that decreased leaf sheath thickness by 45%, while Acetamide showed a 26% decrease in this trait as compared to the untreated control (Figure 3 and Supplementary Figure S3). Epidermal thickness increased only in post-emergence Metolachlor-Atrazine, where a 25% increase was recorded. The maximum decrease in epidermal thickness was observed in pre-emergence Acetamide and post-emergence Methyl ester, showing a 75% decrease as compared to the control. Post-emergence Mesotrione and Atrazine-Mesotrione-Halosulfuron methyl revealed a 50% decrease in this attribute. Cortical cell area generally increased in herbicide-treated plants; the maximum increase was noted in post-emergence Methyl ester (303%). Pre-emergence Acetamide depicted a 256% increase, while pre-emergence Bromoxynil depicted a 222% increase as compared to the control. A significant decrease (60%) in this trait was noticed only in Atrazine-Mesotrione-Halosulfuron methyl-treated plants. Sclerenchymatous thickness invariably decreased with herbicide treatments; the most affected was the pre-emergence Acetamide, where a 60% decrease over the control was recorded (Figure 3). Vascular bundle area was severely affected by all herbicide treatments; the least effective was pre-emergence Bromoxynil showing a decrease of 56%. All other herbicides showed a decrease from 82 to 91% in vascular bundle area as compared to control (Supplementary Figure S3).

3.1.5. Leaf Blade Anatomy

Midrib thickness increased in three treatments, with the maximum increase (25%) as recorded in Acetamide-treated plants (Figure 4 and Supplementary Figure S3). Mesotrione and Atrazine-Mesotrione-Halosulfuron methyl increased midrib thickness by 21 and 13%, respectively. This attribute decreased by 18% in Metolachlor-Atrazine and Methyl ester. Lamina thickness was increased by one herbicide only that resulted in a 26% increase. Two pre-emergence herbicides (Acetamide and Bromoxynil) and one post-emergence Atrazine-Mesotrione-Halosulfuron methyl did not alter lamina thickness. Metolachlor-Atrazine resulted in a decrease of 31%, while Methyl ester exhibited 44% decrease in lamina thickness as compared to the untreated control. Epidermal thickness increased significantly in all herbicide treatments, where,the maximum increase was found in pre-emergence Acetamide and post-emergence Atrazine-Mesotrione-Halosulfuron methyl both showing a 200% increase. All other herbicides resulted in an increase of 100% as compared to the untreated control. Methyl ester exclusively decreased the cortical cell area of *D. aegyptium* leaves, where a 42% decrease was observed. All other herbicides increased this attribute, and the maximum increase (153%) was seen in Mesotrione-treated plants. A variable response was recorded for mesophyll thickness that decreased in three herbicides and increased in the other three. The maximum increase (32%) was noted in Metolachlor-Atrazine, while a 24–28% decrease was noted in Mesotrione- and Atrazine-Mesotrione-Halosulfuron methyl-treated plants. Mesophyll cell area was invariably decreased by herbicide treatment in the leaves of *D. aegyptium*. The maximum decrease was observed in Methyl ester- and Mesotrione-treated plants showing a 59–63% decrease. Vascular bundle area responded positively to all herbicide treatments, as this attribute increased in all treatments. The maximum increase was recorded in Metolachlor-Atrazine-treated plants showing a 113% increase. Pre-emergence Bromoxynil and post-emergence Mesotrione increased this trait by 95 and 103%, respectively (Supplementary Figure S3), over control. Metaxylem area increased by all herbicides except post-emergence Atrazine-Mesotrione-Halosulfuron methyl, where a 35% decrease was noted. The maximum increase was recorded in Methyl ester-treated plants (436%), followed by Metolachlor-Atrazine (344%); both of these are post-emergence herbicides. Two herbicides, Bromoxynil and Metolachlor-Atrazine, increased phloem area by 16–17%, while all other herbicides decreased this parameter. The maximum decrease (66%) was noted in Mesotrione-treated plants.

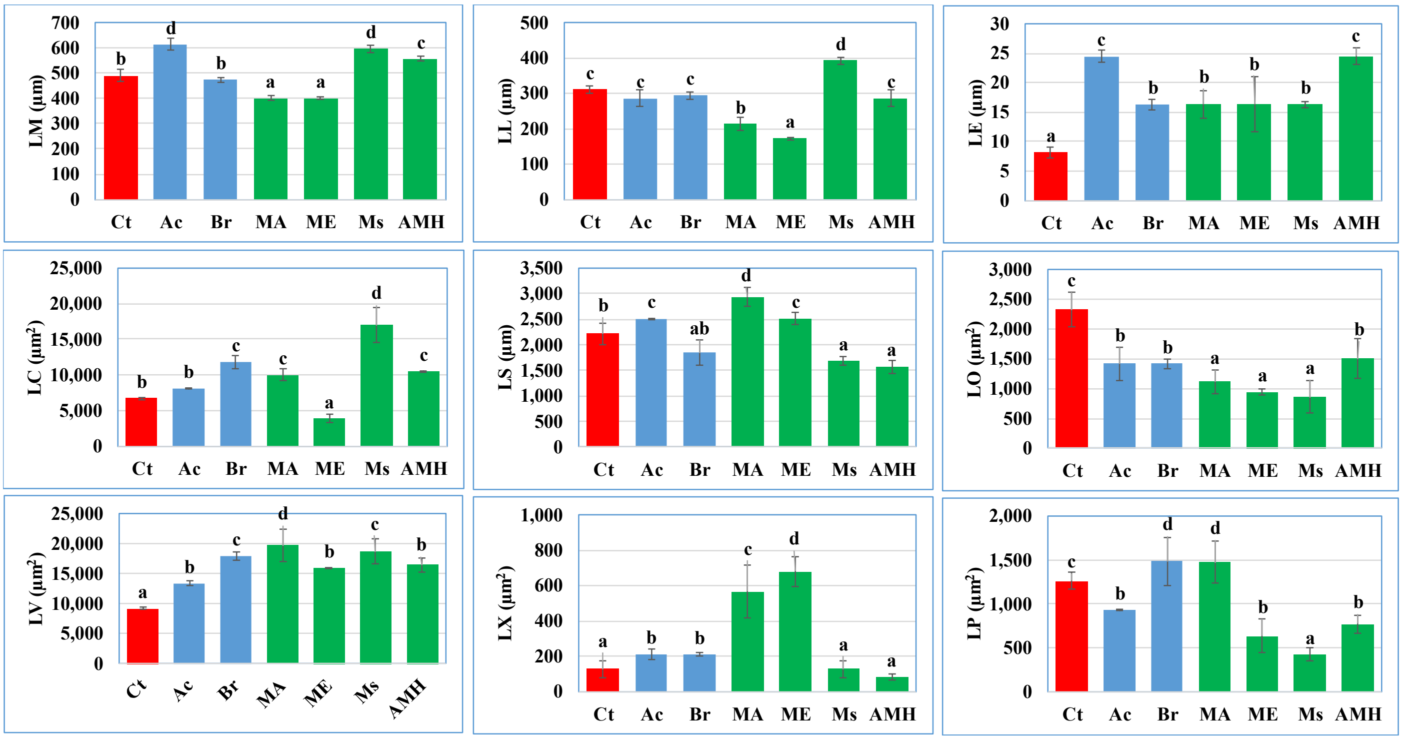
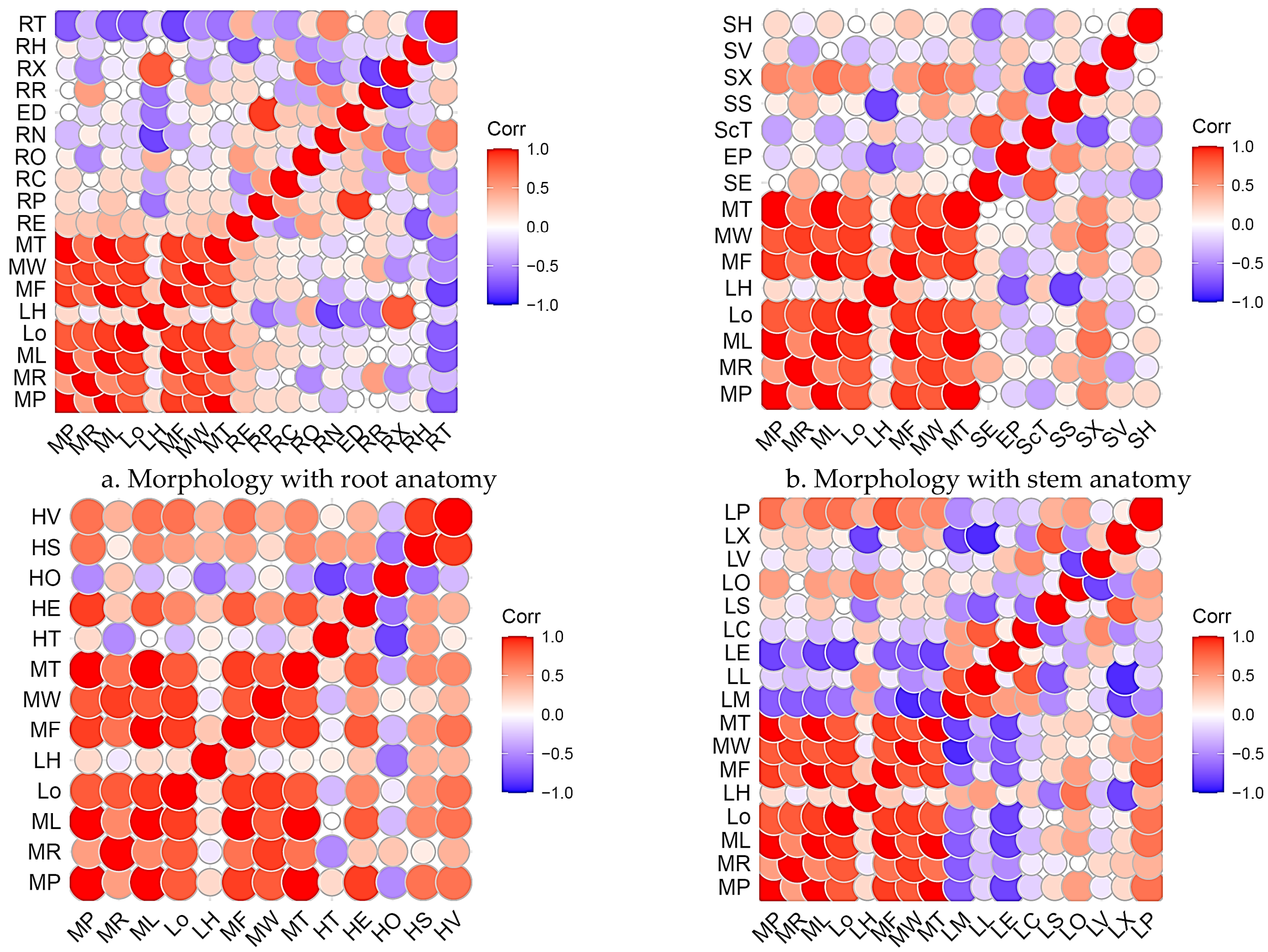


Figure 4. Leaf blade anatomical characteristics of Dactyloctenium aegyptium treated with pre- and post-emergence herbicides. Means sharing same lowercase letters on bars are statistically non−significant at $p \leq 0.05\%$). Red bar indicates control group, blue bars show pre-emergence and green bars are used for post-emergence herbicides. For abbreviations please see list at the end of manuscript.

3.2. Pearson's Correlation Coefficient

Pearson's coefficient correlation between morphological and root anatomical characteristics showed a significant association among all morphological characteristics. Root pith area was negatively correlated with the plant height, leaf area, leaves per plant, and root fresh weight, while root phloem area was negatively correlated with root attributes such as epidermal thickness, metaxylem area, cortical cell area and endodermal thickness with leaf sheath length. A strong positive correlation was recorded between root metaxylem area, leaf sheath length, root endodermal cell area, and root epidermal cell area (Figure 5a). Morphology and stem anatomical characteristics revealed significant morphological characteristics where leaf sheath length was negatively correlated with stem epidermal cell area and stem sclerenchymatous thickness, while stem metaxylem area was negatively correlated stem cortical cell area (Figure 5b). For leaf sheath anatomical attributes, Pearson's coefficient correlation displayed a significant relationship among all morphological attributes. A positive correlation of leaf sheath epidermal thickness was observed with plant height, leaf area, root fresh weight and stem fresh weight (Figure 5c). Pearson's correlation coefficient between morphological and leaf anatomical characteristics (Figure 5d) showed that all morphological characteristics except leaf sheath length showed significant positive correlations with each other (Figure 5d). Leaf sheath length was not correlated with any morphological or leaf anatomical trade except leaf mesophyll thickness and leaf metaxylem area, where a strong negative correlation was observed. A negative correlation between midrib thickness and leaf epidermal thickness was observed. Phloem area was positively correlated with all morphological characters.



a. Morphology with root anatomy

b. Morphology with stem anatomy

c. Morphology with leaf sheath anatomy

d. Morphology with leaf blade anatomy

Figure 5. Pearson's correlation coefficients computed for morphology with root (**a**), stem (**b**), leaf sheath (**c**) and leaf blade (**d**) anatomy of *Dactyloctenium aegyptium* treated with pre- and post-emergence herbicides. For abbreviations please see list at the end of manuscript.

3.3. Relationship between Micro–Structural and Morphological Adaptations with Herbicides

The second question, i.e., the relationship between micro-structural and morphological adaptations with the chemical herbicides was addressed by running a redundancy analysis separately for root, stem, leaf, and leaf sheath. The RDA triplot between morphological and root anatomical characteristics in *D. aegyptium* treated with different post- and pre-emergence herbicides is presented in Figure 6a. Bromoxynil influenced phloem area, which was associated with root length leaf fresh weight. Metolachlor-Atrazine was linked to cortical region thickness and epidermal cell area, which influenced plant height, leaves per plant, leaf area, root fresh weight, and stem fresh weight. Leaf sheath length was associated with the control, and this influenced endodermal cell area, cortical cell area, and metaxylem area. Relationship between morphology and stem anatomical attributes presented an association of the control and Methyl ester with metaxylem area, which influenced leaf area (Figure 6b). Metolachlor-Atrazine was related to epidermal cell area, sclerenchymatous thickness, and phloem area, which influenced plant height and stem fresh weight. Acetamide was associated with leaves per plant, root length, root fresh weight, and leaf fresh weight, but not related to any anatomical attribute.

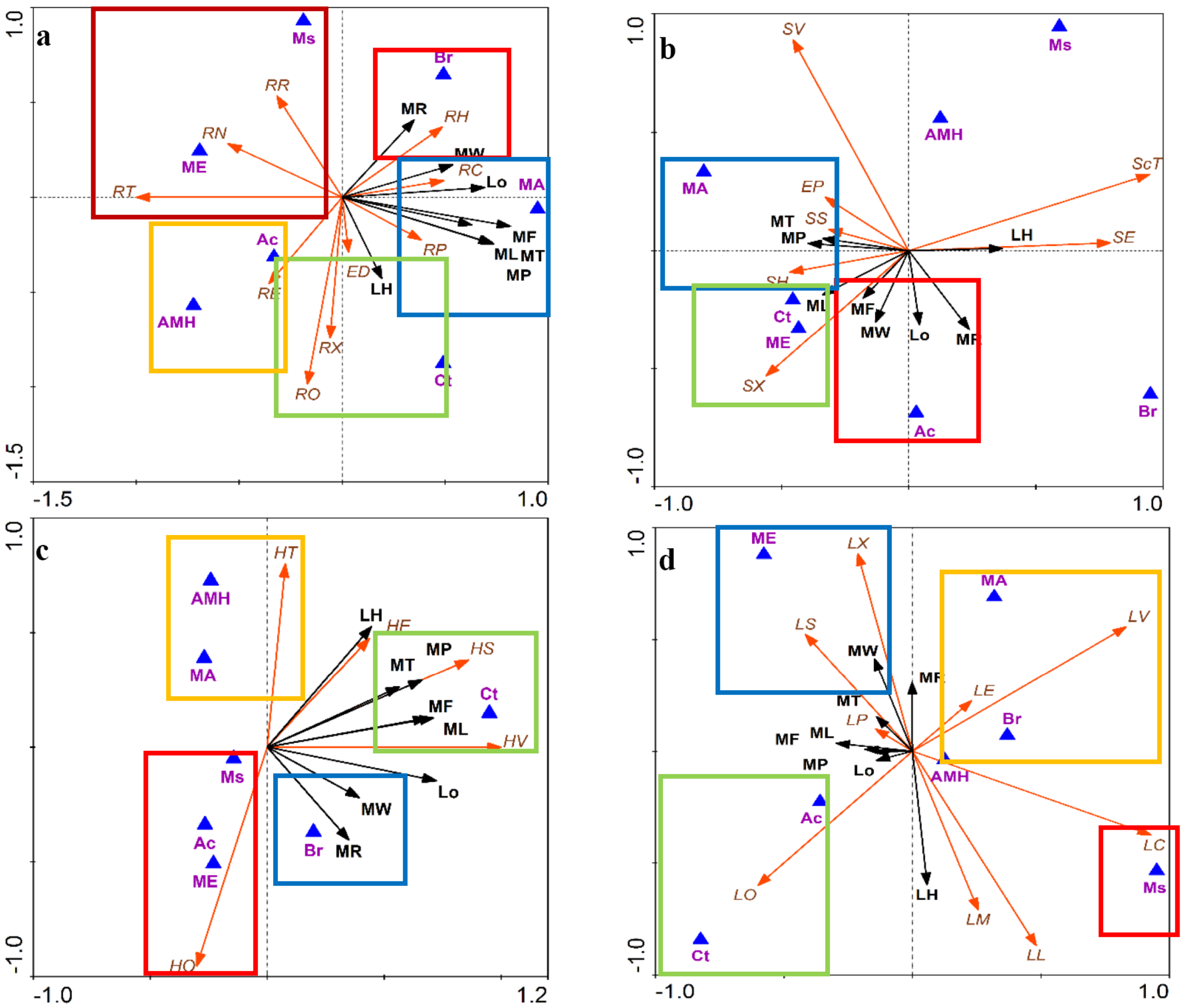


Figure 6. RDA triplot showing the response of morpho-anatomical characteristics in *Dactyloctenium aegyptium* to pre- and post-emergence herbicides. (**a**). Morphology and root anatomy, (**b**). Morphology and stem anatomy, (**c**). Morphology and leaf sheath anatomy, (**d**). Morphology and leaf blade anatomy. The colored rectangles represent grouping of variables with particular herbicides. For abbreviations please see list at the end of manuscript.

Relationship between morphology and leaf sheath anatomical attributes depicted association of control with sclerenchymatous thickness and vascular bundle area (Figure 6c), which influenced plant height, leaf area, root fresh weight and stem fresh weight. Metolachlor-Atrazine and Atrazine-Mesotrione-Halosulfuron methyl were associated with leaf sheath thickness, while Mesotrione, Acetamide and Methyl ester with cortical cell area. However, all these not influenced morphological traits. Redundancy analysis between morphology and leaf anatomical attributes is presented in Figure 6d. Methyl ester was associated with mesophyll thickness and metaxylem area, and these influenced the leaf fresh weight. Other herbicides showed no association with morphological traits. Bromoxynil was associated with epidermal thickness and vascular bundle area, Mesotrione with cortical cell area, and Re and Control with mesophyll cell area.

3.4. Specificity of Herbicide Induced Micro–Structural Modifications

The heatmap between morphological and root anatomical traits showed a grouping of herbicides into two main clusters (Figure 7a). Cluster 1 showed grouped Atrazine-Mesotrione-Halosulfuron, Mesotrione, and Acetamide, while Metolachlor-Atrazine, Control and Bromoxynil were closely grouped in cluster 2. Methyl ester showed an isolated response and was not clustered with any other herbicide. All morphological characteristics showed close association in cluster 1, which formed three sub clusters, where root length and leaf fresh weight, stem fresh weight, plant height and leaf area, mesophyll cell area and leaf epidermal thickness were strongly associated. Root attributes such as cortical region thickness, phloem area, epidermal cell area, endodermal cell area, and endodermal thickness were grouped in cluster 2, while root pith area, leaf sheath length, root metaxylem area, root epidermal thickness and root cortical cell area formed cluster 3 (Figure 7a). Clustered heatmap between morphological and stem anatomical characteristics displayed three distinct grouping of herbicides. Group 1 depicted an association between Mesotrione, Acetamide and Atrazine-Mesotrione-Halosulfuron, group 2 Methyl ester and Metolachlor-Atrazine, while group 3 included Control and Bromoxynil. For stem anatomical traits, in cluster 1 stem metaxylem area was closely associated with all morphological characteristics. Clustered 2 showed association between stem attributes such as phloem area, vascular bundle area, epidermal cell area, and sclerenchymatous thickness, while leaf sheath length, stem epidermal thickness, and stem cortical cell area were grouped in clustered 3 (Figure 7b). Heatmap clustering between morphological and leaf sheath anatomical characteristics grouped herbicides into three distinct clusters (Figure 7c). The cluster 1 showed association between Acetamide, Mesotrione and Atrazine-Mesotrione-Halosulfuron, while Methyl ester and Bromoxynil (cluster 2), and, Mesotrione, Acetamide and Control (cluster 3) were closely associated.

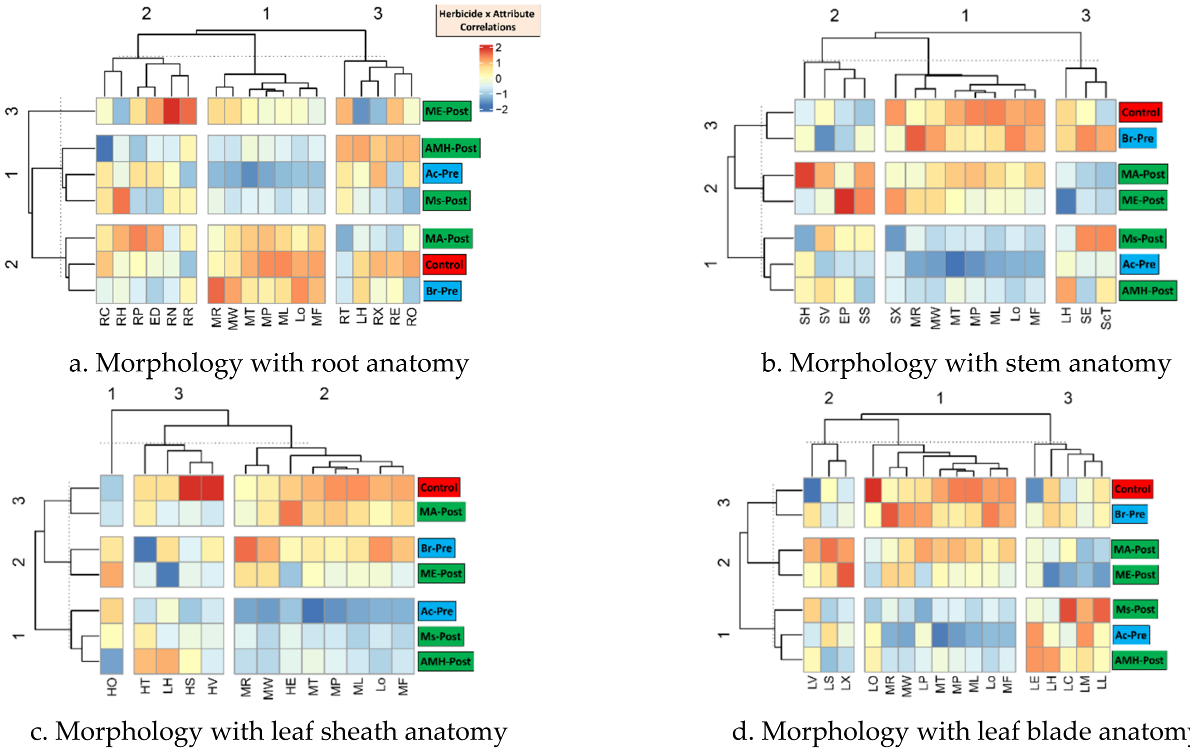


Figure 7. Clustered heatmaps of morphology with root (**a**), stem (**b**), leaf sheath (**c**) and leaf blade (**d**) anatomy of *Dactyloctenium aegyptium* treated with pre- and post-emergence herbicides. Red color indicates control group, blue show pre-emergence and green is used for post-emergence herbicides. For abbreviations please see list at the end of manuscript.

Among leaf sheath attributes, cortical cell area showed isolated behavior since it was not clustered with any other morphological or leaf sheath anatomical characteristics. All morphological characteristics were grouped in cluster 2, while leaf sheath thickness, leaf sheath length, leaf sheath sclerenchymatous thickness, and leaf sheath vascular bundle area were closely grouped (cluster 3). Clustered heatmap plotted between morphological and leaf anatomical attributes showed grouping of herbicides into three distinct clusters

(Figure 7d). The cluster 1 grouped control and Bromoxynil, cluster 2 grouped Metolachlor-Atrazine and Methyl ester and cluster 3 group Mesotrione, Acetamide and Atrazine-Mesotrione-Halosulfuron methyl. A large group of leaf anatomical attributes were grouped in cluster 1, where a close association between root fresh weight and leaves per plant, stem fresh weight, plant height, leaf area, root length and leaf fresh weight was observed. Characteristics such as leaf vascular bundle area, mesophyll thickness and metaxylem area were closely grouped in cluster 2, whereas leaf attributes such as epidermal thickness, cortical cell area midrib thickness, and lamina thickness showed strong association in cluster 3.

3.5. Principle Component Analysis for Assessment of Mode of Application and Herbicide Specific Effects

Principle component analysis based on herbicides indicated four distinct clusters. Post-emergence Atrazine-Mesotrione-Halosulfuron methyl showed association with sheath thickness, leaf cortical cell area, leaf sheath length, lamina thickness, midrib thickness, root pith area, root phloem area, root metaxylem area, stem epidermal thickness and stem cortical cell area. Pre-emergence Acetamide and post-emergence Mesotrione were related to leaf epidermal thickness. Post-emergence Metolachlor-Atrazine and Methyl ester exhibited relationship with root endodermal cell area, stem epidermal cell area, sheath cortical cell area, mesophyll thickness, leaf metaxylem area, root cortical region thickness, root epidermal cell area, pericycle thickness, endodermal thickness and sclerenchymatous thickness. Control and pre-emergence Bromoxynil were associated with leaf sheath traits vascular bundle area, epidermal thickness, sclerenchymatous thickness, morphological traits leaves per plant, root fresh weight, leaf area, plant height, stem fresh weight, root length, leaf mesophyll cell area, stem phloem area and stem metaxylem area (Figure 8).

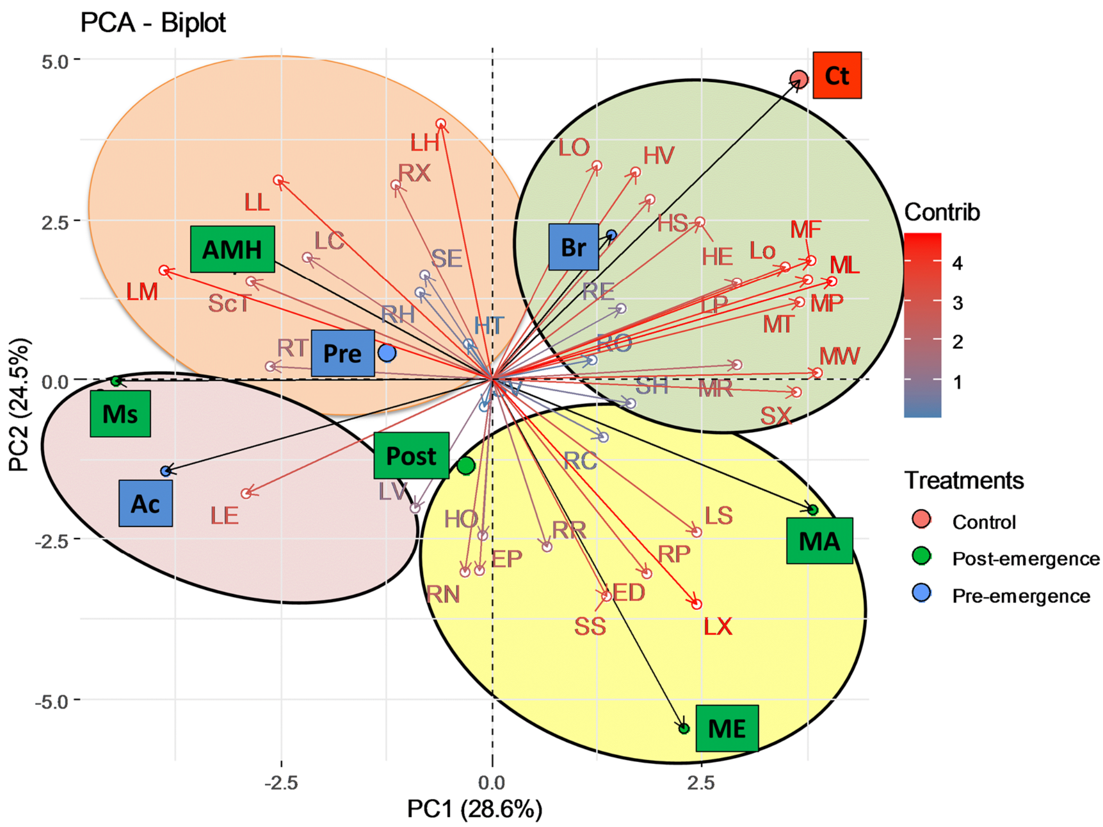


Figure 8. Principal component analysis representing relationship of various morphological and anatomical characteristics of *Dactyloctenium aegyptium*. The eclipses are drawn to group parameters

on the basis of herbicides and mode of application. Red color indicates control group, blue show pre-emergence and green is used for post-emergence herbicides. For abbreviations please see list at the end of manuscript.

Principal component analysis based on mode of application revealed two distinct groups (Figure 8). Pre-emergence herbicides showed association with large number of attributes such as midrib thickness, lamina thickness, metaxylem area, leaf sheath length, mesophyll cell area, sheath vascular bundle area, sheath sclerenchymatous thickness, sheath epidermal cell area, leaves per plant, root fresh weight, leaf phloem area, leaf area, plant height, stem fresh weight and leaf fresh weight. Post-emergence herbicides indicated association with root endodermal thickness, stem epidermal cell area, stem sclerenchymatous thickness, root pericycle thickness, root endodermal cell area, mesophyll thickness, leaf metaxylem area and root epidermal cell area. Traits such as stem cortical cell area, leaf cortical cell area, root pith area, leaf epidermal thickness, root phloem area, stem epidermal thickness, sheath epidermal thickness, stem vascular bundle area, root cortical cell area, stem phloem area, root cortical region thickness, root length and stem metaxylem area were influenced by pre-emergence and post-emergence herbicides.

3.6. Clustering of Herbicides Treatments Based on the Observed Plant Growth Traits

Cluster analysis based on various morpho-anatomical attributes showed two groups of clusters, the first included Control, Bromoxynil and Metolachlor-Atrazine, and the other had association among Acetamide, Mesotrione, Methyl ester and Atrazine-Mesotrione-Halosulfuron. A strong association of post-emergence herbicides Methyl ester and Atrazine-Mesotrione-Halosulfuron was recorded (Figure 9). Pre-emergence herbicide Acetamide closely clustered with post-emergence Mesotrione, while pre-emergence Bromoxynil cultured with Control.

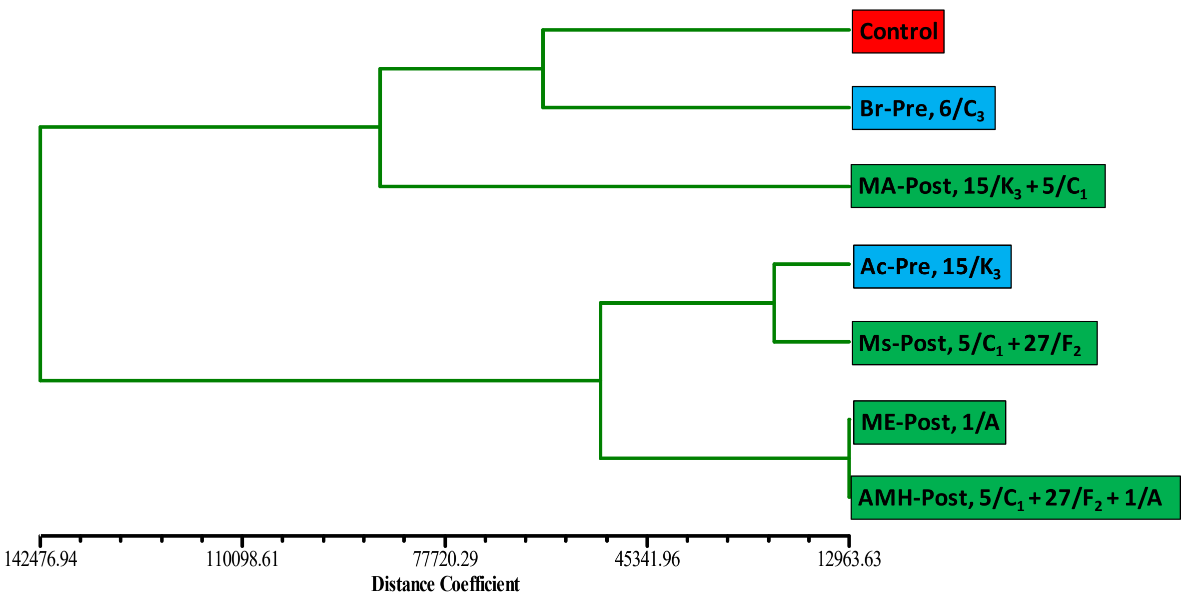


Figure 9. Unweighted pair group method with arithmetic mean cluster analysis using the sequential agglomerative hierarchical non-overlapping method of different herbicides based on collective morpho-anatomical attributes. Mode of action: The numeric are classified by Weed Science Society of America, K3-Inhibition of cell cycle, C3 and C1-Inhibition of photosynthesis in photosystem II, A-Inhibition of Acetyl-CoA Carboxylase (ACCase), F2-Inhibition of carotenoid biosynthesis in 4-hydroxyphenyl-pyruvate-dioxygenase (4HPPD). Red color indicates control group, blue show pre-emergence and green is used for post-emergence herbicides. For abbreviations please see list at the end of manuscript.

3.7. Classification of Tolerance Mechanisms/Alterations by Mode of Application

Based on mode of herbicide action, post-emergence Atrazine-Mesotrione-Halosulfuron and Methyl ester being acetyl CoA carboxylase inhibitor (1/A) were closely clustered (Figure 9). The ACCase catalyzes the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA and is the first committed step in fatty acid synthesis. This showed the capacity of both these herbicides to inhibit fatty-acid biosynthesis and may cause weed death due to depletion of fatty acids. Another post emergence herbicide Mesotrione was distantly sub-clustered with the above group (containing Atrazine-Mesotrione-Halosulfuron and Methyl ester) in the main cluster.

Both these herbicides cause inhibition of photosystem-II of photosynthetic machinery (5/C1) and inhibition of carotenoid biosynthetic pathway in 4-hydroxyphenyl pyruvate deoxygenase (27/F2) as common toxicity mechanisms. Such herbicide induced effects cause a substantial inhibition of photosynthetic pathways thereby restricting weed growth. In the same cluster, a pre-emergence herbicide (Acetamide) which is an inhibitor of cell cycle (15/K3), was grouped distantly perhaps due to differences in toxicity mechanism. In the second cluster, either a pre-emergence herbicide Bromoxynil or post emergence herbicide (Metolachlor-Atrazine) were clustered together. Regardless of the differences in mode of application, both these herbicides share 5/C1 and 6/C3 (inhibition of photosystem-II of photosynthetic machinery of weeds) as the same toxicity mechanism.

4. Discussion

Overall response of different post- and pre-emergence herbicides is presented in Supplementary Figure S4. Growth and development regarding plant height, leaves per plant and leaf area, root, and stem fresh weights of *D. aegyptium* were the best in the untreated control. Prominent features in root were the thicker epidermis, cortical parenchyma and broader metaxylem vessels. Root epidermis controls the uptake of water and nutrients [14], while cortical region thickness along with large cortical cell was related to storage capacity of roots. Moreover, broader vessels enhance the conduction of solutes from roots to aboveground plant parts, resulting in growth enhancement [15]. Other anatomical attributes minutely contributed in the growth and development of the untreated control plants. Stem metaxylem vessels and leaf mesophyll area was the maximum, which is the indication of their involvement in better growth and development.

Dactyloctenium aegyptium plants though survived when treated with pre-emergence Acetamide, but growth and development was badly hampered. Anatomical attributes were also affected, where the positive response was recorded for root cortical cell area and metaxylem area, leaf epidermal thickness, and most importantly leaf thickness that is mainly due to mesophyll thickness. Herbicide Acetamide inhibits protein and lipid synthesis [16], and effects other metabolic processes such as photosynthesis [17]. Pre-emergence Bromoxynil enhanced root growth and root length. The only prominent feature among anatomical attributes was the increased stem epidermal thickness. Epidermis is a protective layer that is in direct contact with external environments [18]. Herbicides have to pass through epidermis to interact with plant structural and functional features [19] and thicker epidermis offers defense against the penetration of herbicides [20].

Post-emergence Metolachlor-Atrazine showed greater root and stem fresh weights than other herbicides along with number of root, stem and leaf anatomical characteristics. In roots, epidermal cell area, endodermal cell area and phloem area were the maximum, while epidermal thickness, endodermal thickness and pith area were the minimum. Epidermal and endodermal cells changed their shape from drum-shaped to a more elongated shape, it is known that thinner protective layers are less prone to herbicide penetration [21]. Stem sclerenchymatous thickness, vascular bundle area and phloem area were the maximum in Metolachlor-Atrazine-treated plants. Sclerenchyma formation is the immediate response of plants to counteract water shortage due to herbicide application by protecting plants from mechanical injury [22] and also minimizing water loss through plant surface [23]. Larger vascular bundles are linked to better conduction of water, nutrients and photosynthates, hence improving the survivability of *D. aegyptium* plants treated with Metolachlor-Atrazine. Leaf characteristics such as mesophyll thickness, vascular bundle area and phloem area were the maximum. Mesophyll is the photosynthetically active tissue that produces energy for growth and survival of Metolachlor-Atrazine-treated plants.

Methyl ester, a post-emergence herbicide exhibited negative impact on growth and germination of *D. aegyptium* plants, however there was significant decrease in leaf sheath length. Root anatomical attributes such as epidermal thickness, endodermal thickness and endodermal cell area were the maximum, which control influx and radial movements of solute in the roots [24]. The narrowest metaxylem vessels are beneficial in a way that they are less likely to be damaged by collapse and cavitation, as herbicide treatment can also cause water scarcity [25]. Stem traits such as epidermal cell area, sclerenchyma thickness and metaxylem area were the maximum in Methyl ester-treated plants, and this might be the good reason for survival of *D. aegyptium*. Epidermis and sclerification of stem peripheral region collectively are vital modifications for the prevention of herbicide penetration in the Methyl ester-treated plants [26]. Cortical cell area in the leaf sheath increased significantly, which was associated with better storage capacity [7], so increasing the survival chances in Methyl ester-treated plants. Vascular bundle area in leaf sheath decreased but metaxylem area in leaf blade was the maximum. Broader metaxylem vessels offer less tolerance to solute conduction in *D. aegyptium* [27].

Mesotrione application did not alter significantly morphological and leaf sheath anatomical attributes. Root epidermal and endodermal thicknesses, epidermal, endodermal and cortical cell area were the maximum in Mesotrione-treated plants. This crucial modification tolerates herbicide penetration in roots and additionally provides better water storage capacity [28]. Epidermal thickness and cortical cell area in stem increased significantly that was vital for the survival against Mesotrione application [29]. Thicker leaves were primarily due to the cortical parenchyma, and the high proportion of storage parenchyma is extremely beneficial when plants are exposed to environmental adversaries. This is a characteristic feature under water deficit condition as herbicide application can cause water shortage in plants, which are relatively less tolerant [30].

Atrazine-Mesotrione-Halosulfuron methyl, a post-emergence herbicide, increased epidermal thickness and root area. It also increased leaf sheath length and thickness and caused extensive sclerification along with thickening the epidermis in leaf blade. All these aspects increase tolerance to herbicide penetration, water storage capacity and confer mechanical support to soft and delicate inner tissues [31]. A number of root, stem and leaf anatomical characteristics were severely affected by Atrazine-Mesotrione-Halosulfuron methyl application. These included root dermal and parenchymatous tissue, stem dermal and sclerenchymatous tissue, leaf sheath parenchymatous and vascular tissue, and leaf mesophyll and metaxylem vessels. All these modifications will negatively influence radial water movement [32], water and nutrient storage photosynthetic capacity [33] and conduction of solutes. Collectively, these structural alterations will result in severe damages to growth and development under herbicide treatment applications [34].

Table 1. Generic names and mode of action of various pre- and post-emergence herbicides sprayed on *Dactyloctenium aegyptium* (L.) Willd.

Herbicide Name	Efficacy on *D. aegyptium* (%)	Rate Applied	Mode of Action	Cellular Processes Affected
Pre-emergence herbicides				
Acetamide (Ac) [2-chloro-N-(2-ethyl-6-methylph enyl)acetamide]	41.7 ± 3	2250 ml ha^{-1}	15/K_3	Inhibits protein, lipid and terpenoids synthesis [16] and other metabolic processes like photosynthesis, respiration, RNA synthesis, etc. [17].
Bromoxynil (Br) [3,5-dibromo-4-hydroxybenzoni trile]	25.8 ± 2	2250 mL ha^{-1}	6/C_3	Uncouples oxidative phosphorylation [35].
Post emergence herbicides				
Metolachlor-Atrazine (MA) [720 SC [290 g LS-Metolachlor + 370 g LAtrazine + Shift 37% OD] [Pretilachlor 50% EC]	30.5 ± 3	2000 mL ha^{-1}	15/K_3 + 5/C_1	Herbicide is inhibitor of photosynthesis at photosystem II [36].
Methyl ester (ME) [Methyl 3-chloro-5-(4,6-dimethoxypyrim id- in-2-ylcarbamoylsulfamoyl)-1-m ethylpyrazole-4-carboxylate]	38.9 ± 4	50 g ha^{-1}	1/A	Inhibits acetolactate synthetase (ALS) enzymes, thus arrests growth [37].
Mesotrione (Ms) [2-[4-(methylsulfonyl)-2-nitrobe nzoyl]-1,3-cyclohexanedione]	31.7 ± 2	2000 mL ha^{-1}	5/C_1 + 27/F_2	Inhibits photosynthesis through inhibiting electron transport of photosystem II [38].
Atra-zine-Mesotrione-Halosulfuron methyl (AMH)	28.2 ± 1	2000 ml ha^{-1}	5/C_1 + 27/F_2 + 1/A	It is a photosynthesis inhibitor which inhibits electron transport of photosystem II, leading to inhibition of photosynthesis [38,39].

The numeric in mode of action column are classified by Weed Science Society of America, K3-Inhibition of cell cycle, C3 and C1-Inhibition of photosynthesis in photosystem II, A-Inhibition of Acetyl-CoA Carboxylase (ACCase), F2-Inhibition of carotenoid biosynthesis in 4-hydroxyphenyl-pyruvate-dioxygenase (4HPPD).

5. Conclusions

Dactyloctenium aegyptium showed significantly different responses under pre-emergence and post emergence herbicides application. Morphological, anatomical, and physiological features help survival against herbicides. Pre-emergence herbicide Bromoxynil enhanced root length and leaf fresh weight as compared to the control. The only prominent feature among anatomical attributes was the increased stem epidermal thickness. Epidermis is a protective layer that is in direct contact with external environments. Post-emergence herbicide increased leaf sheath length, epidermal thickness, leaf sheath thickness, root area in roots, sclerenchymatous thickness in leaf sheath, and epidermal thickness in leaf blade. Thicker leaves having high proportion of storage parenchyma are very helpful when a plant is exposed to herbicide application. These characters may assist *D. aegyptium* species to adjust herbicide toxicity. These features confirmed the differential responses of this weed under a broad range of pre- and post-emergence herbicides. Growth and morphological characteristics were severely affected by the application of pre-emergence Acetamide, which inhibited proteins, lipids, and terpenoids synthesis, and affected metabolic processes such as photosynthesis, respiration, and RNA synthesis. Post-emergence Atrazine-Mesotrione-Halosulfuron methyl affected anatomical traits more severely than other herbicides, which is a photosynthesis inhibitor. It is suggested that these two herbicides should collectively be applied for the affective control of *D. aegyptium.*

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12111831/s1, Figure S1: Layout plan of the application of different pre- and post-emergence herbicides on *Dactyloctenium aegyptium*; Figure S2. Response of root and stem anatomical characteristics of *Dactyloctenium aegyptium* to pre- and post-emergence herbicides. Figure S3. Response of leaf sheath and leaf blade anatomical characteristics of *Dactyloctenium aegyptium* to pre- and post-emergence herbicides. Figure S4. Proposed model of the overall impact of pre- and post-emergence herbicides on morpho-anatomical attributes of *Dactyloctenium aegyptium.*

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Abbreviations

Herbicides:	Br- Bromoxynil: Ct-Control: AMH-Atrazine-Mesotrione-Halosulfuron methyl, Ms-Mesotrione, ME-Methyl ester, MA-Metolachlor-Atrazine, Ac-Acetamide
Application Mode:	Pre-Pre-emergence, Post-Post-emergence.
Morphology:	Lo-Leaves per plant, LH-Leaf sheath length, MD-Root dry weight, MF-Root fresh weight, ML-Leaf area, MP-Plant height, MR-Root length, MT-Stem fresh weight, MU-Stem dry weight, MW-Leaf fresh weight, MX-Leaf dry weight
Root anatomy:	ED-Endodermal cell area, EP-Epidermal cell area, RC-Cortical region thickness, RD-Root radius, RE-Epidermal thickness, RH-Phloem area, RN-Endodermal thickness, RO-Cortical cell area, RP-Epidermal cell area, RR-Pericycle thickness, RT-Pith area, RX-Metaxylem area
Stem anatomy:	ScT-Cortical cell area, SC-Epidermal cell area, SE-Epidermal thickness, SH-Phloem area, SR-Stem radius, SS-Sclerenchymatous thickness, SV-Vascular bundle area, SX-Metaxylem area
Leaf sheath anatomy:	HE-Epidermal thickness, HO-Cortical cell area, HS-Sclerenchymatous thickness, HT-Sheath thickness, HV-Vascular bundle area
Leaf blade anatomy:	LC-Cortical cell area, LE-Epidermal thickness, LL-Lamina thickness, LM-Midrib thickness, LO-Mesophyll cell area, LP-Phloem area, LSL-Leaf sheath length, LS-Mesophyll thickness, LV-Vascular bundle area, LX-Metaxylem area

References

1. Lawrence, B.H.; Bond, J.A.; Edwards, H.M.; Golden, B.R.; Montgomery, G.B.; Eubank, T.W.; Walker, T.W. Effect of fall-applied residual herbicides on rice growth and yield. *Weed Technol.* **2018**, *32*, 526–531.
2. Naseer-ud-Din, G.M.; Shehzad, M.A.; Nasrullah, H.M. Efficacy of various pre and post-emergence herbicides to control weeds in wheat. *Pak. J. Agric. Sci.* **2011**, *48*, 185–190.
3. Qasem, J.R. Herbicide resistant weeds: The technology and weed management. *Herbicides—Current Research and Case Studies in Use*; InTech Publishers: London, UK, 2013; pp. 445–471.
4. Powles, S.B.; Yu, Q. Evolution in action: Plants resistant to herbicides. *Annu. Rev. Plant Biol.* **2010**, *61*, 317–347.
5. Vila-Aiub, M.M.; Balbi, M.C.; Distéfano, A.J.; Fernández, L.; Hopp, E.; Yu, Q.; Powles, S.B. Glyphosate resistance in perennial *Sorghum halepense* (Johnsongrass), endowed by reduced glyphosate translocation and leaf uptake. *Pest. Manag. Sci.* **2012**, *68*, 430–436.

6. Huangfu, C.; Song, X.; Qiang, S. Morphological disparities in the epidermal and anatomical features of the leaf among wild *Brassica juncea* populations. *Weed Biol. Manag.* **2009**, *9*, 234–242.
7. Cobb, A.H.; Reade, P.H.J. *Herbicides and Plant Physiology*, 2nd ed.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2011.
8. Perotti, V.E.; Larran, A.S.; Palmieri, V.E.; Martinatto, A.K.; Permingeat, H.R. Herbicide resistant weeds: A call to integrate conventional agricultural practices, molecular biology knowledge and new technologies. *Plant Sci.* **2020**, *290*, 110255.
9. Khaliq, A.; Matloob, A.; Ahmad, N.; Rasul, F.; Awan, I.U. Post emergence chemical weed control in direct seeded fine rice. *J. Anim. Plant Sci.* **2012**, *22*, e1106.
10. Sanglakpam, P.; Mathur, R.R.; Pandey, A.K. Ethnobotany of Chothe tribes of Bishnupur district (Manipur). *Indian J. Nat. Prod. Res.* **2012**, *3*, 414–425.
11. Tauseef, M.; Ihsan, F.; Nazir, W.; Farooq, J. Weed flora and importance value index (IVI) of the weeds in cotton crop fields in the region of Khanewal, Pakistan. *Pak. J. Weed Sci. Res.* **2012**, *18*, 319–330.
12. Rohlf, F.J. NTSYS-pc: *Numerical Taxonomy and Multivariate Analysis System*, version 2.2; Exeter Publications: Setauket, NY, USA, 2000.
13. Yim, O.; Ramdeen, K.T. Hierarchical cluster analysis: Comparison of three linkage measures and application to psychological data. *Quant. Methods Psychol.* **2015**, *11*, 8–21.
14. Barberon, M.; Geldner, N. Radial transport of nutrients: The plant root as a polarized epithelium. *Plant Physiol.* **2014**, *166*, 528–537.
15. Blum, A. Plant water relations, plant stress and plant production. In *Plant Breeding for Water–Limited Environments*; Blum, A., Ed.; Springer: New York, NY, USA, 2011; pp. 11–52.
16. Hess, F.D. Herbicide effects on plant structure, physiology, and biochemistry. In *Pesticide Interactions in Crop Production*; Altman, J., Ed.; CRC Press: Boca Raton, FL, USA, 2018; pp 13–34.
17. Fedtke, C. *Biochemistry and Physiology of Herbicide Action*; Springer Science & Business Media: New York, NY, USA; Berlin/Heidelberg, Germany, 2012; Available online: https://link.springer.com/book/10.1007/978-3-642-68375-6 (accessed on 10 September 2022).
18. Lefèvre-Utile, A.; Braun, C.; Haftek, M.; Aubin, F. Five functional aspects of the epidermal barrier. *Int. J. Mol. Sci.* **2021**, *22*, 11676.
19. Krähmer, H.; Walter, H.; Jeschke, P.; Haaf, K.; Baur, P.; Evans, R. What makes a molecule a pre-or a post-herbicide—How valuable are physicochemical parameters for their design? *Pest. Manag. Sci.* **2021**, *77*, 4863–4873.
20. da Cunha Valença, D.; Bezerra, A.C.M.; Ferreira, M.A.; Junqueira, N.E.G.; Macrae, A.; Medici, L.O.; Reinert, F. Changes in leaf blade morphology and anatomy caused by clomazone and saflufenacil in *Setaria viridis*, a model C_4 plant. *S. Afr. J. Bot.* **2020**, *135*, 365–376.
21. Barroso, A.A.M.; Galeano, E.; Albrecht, A.J.P.; Dos Reis, F.C.; Victoria Filho, R. Does sourgrass leaf anatomy influence glyphosate resistance? *Comun. Sci.* **2015**, *6*, 445–453.
22. Lamalakshmi Devi, E.; Kumar, S.; Basanta Singh, T.; Sharma, S.K.; Beemrote, A.; Devi, C.P.; Wani, S.H. Adaptation strategies and defence mechanisms of plants during environmental stress. In *Medicinal Plants and Environmental Challenges*; Ghorbanpour, M., Varma, A., Eds.; Springer: Berlin/Heidelberg, Germany, 2017; pp. 359–413.
23. Abouziena, H.F.; El-Saeid, H.M.; Amin, A.A.E. Water loss by weeds: A review. *Int. J. Chemtech. Res.* **2014**, *7*, 323–336.
24. Meyer, C.J.; Peterson, C.A.; Steudle, E. Permeability of *Iris germanica's* multiseriate exodermis to water, NaCl, and ethanol. *J. Exp. Bot.* **2011**, *62*, 1911–1926.
25. Gianessi, L.P. The increasing importance of herbicides in worldwide crop production. *Pest. Manag. Sci.* **2013**, *69*, 1099–1105.
26. Kraehmer, H.; Baur, P. *Weed Anatomy*; John Wiley & Sons: Hoboken, NJ, USA, 2013.
27. Fatima, S.; Hameed, M.; Ahmad, F.; Ashraf, M.; Ahmad, R. Structural and functional modifications in a typical arid zone species *Aristida adscensionis* L. along altitudinal gradient. *Flora* **2018**, *249*, 172–182.
28. Ghanizadeh, H.; Harrington, K.C. Non-target site mechanisms of resistance to herbicides. *Crit. Rev. Plant Sci.* **2017**, *36*, 24–34.
29. Vrbničanin, S.; Stefanović, L.; Božić, D.; Sarić, M.; Radošević, R. Comparative analysis of the anatomy of two populations of red-root amaranth (*Amaranthus retroflexus* L.). *Pestic. I Fitomedicina* **2009**, *24*, 103–112.
30. Aswani, V.; Rajsheel, P.; Bapatla, R.B.; Sunil, B.; Raghavendra, A.S. Oxidative stress induced in chloroplasts or mitochondria promotes proline accumulation in leaves of pea (*Pisum sativum*): Another example of chloroplast-mitochondria interactions. *Protoplasma* **2019**, *256*, 449–457.
31. Pereira, M.R.R.; Martins, A.R.; Martins, D.; Sasso, G.; Silva, A. Effect of sethoxydim herbicide in the leaf anatomy and physiology of *Brachiaria* grass under water stress. *Planta Daninha* **2017**, 35, e017162268.
32. Schneider, H.M.; Wojciechowski, T.; Postma, J.A.; Brown, K.M.; Lücke, A.; Zeisler, V.; Lynch, J.P. Root cortical senescence decreases root respiration, nutrient content and radial water and nutrient transport in barley. *Plant Cell Environ.* **2017**, *40*, 1392–1408.
33. Sellin, A.; Tullus, A.; Niglas, A.; Ounapuu, E.; Karusion, A.; Lohmus, K. Humidity-driven changes in growth rate, photosynthetic capacity, hydraulic properties and other functional traits in silver birch (*Betula pendula*). *Ecol. Res.* **2013**, *28*, 523–535.
34. Guo, H.; Hu, Z.; Zhang, H.; Min, W.; Hou, Z. Comparative effects of salt and alkali stress on antioxidant system in cotton (*Gossypium hirsutum* L.) leaves. *Open Chem*. **2019**, *17*, 1352–1360.
35. Shimmen, T. Further electrophysiological studies on cellular effect of herbicide, bromoxynil, using characean cells. *J. Plant Res.* **2012**, *125*, 749–754.

36. Beaulieu, M.; Cabana, H.; Huot, Y. Adverse effects of atrazine, DCMU and metolachlor on phytoplankton cultures and communities at environmentally relevant concentrations using Fast Repetition Rate Fluorescence. *Sci. Total. Environ.* **2020**, *712*, 136239.
37. Zhao, B.; Huo, J.; Liu, N.; Zhang, J.; Dong, J. Transketolase is identified as a target of herbicidal substance α-terthienyl by proteomics. *Toxins* **2018**, *10*, 41.
38. Richburg, J.T.; Norsworthy, J.K.; Barber, T.; Roberts, T.L.; Gbur, E.E. Tolerance of corn to PRE-and POST-applied photosystem II–inhibiting herbicides. *Weed Technol*. **2020**, 34, 277–283.
39. Rehan, M.; Sharkawy, A.E.; Fadly, G.E. Microbial biodegradation of S-triazine herbicides in soil. *J. Crop Res. Fert.* **2016**, *1*, 1–6.