*Pak. J. Bot.*, 58(1) January 2026.

ARTICLE | DOI: http://dx.doi.org/10.30848/PJB2026-1(34)

# IMPACT OF PARTHENIUM WEED ON WATER BUDGET, EVAPOTRANSPIRATION, PHOTOSYNTHETIC TRAITS, AND OSMOLYTES IN VEGETABLES: A METHOD DEVELOPMENT APPROACH

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**Abstract**

*Parthenium hysterophorus* is an aggressive invasive weed that severely affects water availability and crop productivity. This study aimed to develop a simplified method for water budget monitoring by evaluating the relationship between key above- and below-ground parameters (canopy cover, biomass, and root-shoot interactions) in 250 parthenium samples. Additionally, the impact of parthenium on winter vegetable crops (broccoli, lettuce, and peas) were assessed under co-cultivation conditions. Physiological responses including biomass production, photosynthetic traits, gas exchange parameters, osmolytes accumulation, and evapotranspiration (ET) were evaluated at three distinct growth stages (14, 25, and 40 days). ET rates were recorded weekly. Results demonstrated that Parthenium significantly increased transpiration rates and crop water consumption, with ETc values estimated using the Penman-Monteith equation showing up to a 30% increase compared to control plants. In contrast, biomass production, photosynthetic activity, and gas exchange parameters of vegetable crops were notably reduced (20–40%) in the presence of parthenium, while osmolytes accumulation increased as a stress response. A fuzzy logic model was successfully applied to enhance water budget prediction accuracy by integrating multiple plant and soil parameters. The study highlights the critical role of parthenium in altering soil moisture dynamics and water-use efficiency in cropping systems.

**Key words:** *Parthenium hysterophorus*; Water budget monitoring; Evapotranspiration; Osmolytes accumulation; Fuzzy logic model

## Introduction

Water deficit is a major challenge in agriculture, particularly in regions that require high-efficiency water management for sustainable crop production (Kang *et al*., 2021). The growing imbalance between rising food demand and insufficient water supply for agriculture is a critical global challenge (Mancosu *et al*., 2015). Agriculture is the largest water-consuming sector, accounting for over 70% of total global water use. As the demand for food continues to increase, expanding agricultural water use alone is not a sustainable solution (Wu *et al*., 2022). Water-stress conditions significantly hinder seed germination, plant growth, development, and seed production. Insufficient water during these critical stages disrupts cellular processes, leading to poor seedling establishment, stunted growth, and reduced reproductive success (Singh *et al*., 2022). In agricultural fields, water availability is influenced not only by crops but also by weed species that compete for essential resources. Weeds reduce soil moisture and increase evapotranspiration (ET), intensifying water stress in crops (Ramesh *et al*., 2017; Kaur *et al*., 2018; Singh *et al*., 2022). The Weed Loss Committee of the Weed Science Society noted that weeds can reduce crop yields by up to 50% in maize (*Zea mays* L.) (Shrinivas, 2016) and 52% in soybean (*Glycine max*) (Soltani *et al*., 2017). In India, weeds contribute to significant yield losses, causing 36% loss in peanut (*Arachis hypogaea* L.) (Priya *et al*., 2013), 31% in soybean, 25% in maize and sorghum (*Sorghum bicolor* L.), and 19% in wheat (*Triticum aestivum* L.) (Gharde *et al*., 2018). Research has shown that maintaining a weed-free period from 20 to 100 days after sowing (DAS) significantly enhances crop performance (Mir *et al*., 2024). In mustard crops, this practice resulted in a 54% increase in dry weight and a 55% improvement in grain yield, while concurrently reducing weed dry weight by up to 20%. Moisture availability significantly influences the competition between millet and weeds (Mishra, 2016). Previous research reported that competition with *H. glaucum*, characterized by high panicle density, and caused yield losses of up to 65% in field pea, highlighting the impact of weed competition on crop productivity under such conditions (Adu-Yeboah, 2021).

*Parthenium hysterophorus*, a highly invasive weed from the Asteraceae family, poses a severe threat to both natural ecosystems and agriculture (Adkins & Shabbir, 2014). The wide spread of this creeper plant in different area global shows its versatility and harm to agriculture and natural habitat (Chhogyel *et al*., 2021). As noted by Kanchan & Jayachandra, 1979; Pandey, 1994, who pointed out that parthenium is among the most notorious invasive plant species globally, which owes this to its great allelopathic capabilities. They have been known to some severely affect agricultural yields: grains and forage crops reducing in yield by 40-90%. The winter crops including the broccoli (*Brassica oleracea*), lettuce (*Lactuca sativa*), and peas (*Pisum sativum*) are of high market essence and nutrient value (Boyhan *et al*., 2016). Weed infestation exhibited a strong negative correlation with the yield parameters of broccoli, indicating that increased weed presence significantly reduced the crop's productivity (Latif *et al*.,

Received: 15-03-2025 | Revised: 22-08-2025 | Accepted: 30-08-2025 | Online: 30-09-2025

2021). The highly invasive nature of *parthenium* disrupts neighboring plants by limiting resource availability, with its physiological impact primarily attributed to its allelopathic properties (Tiawoun *et al*., 2024).

Parthenium alters water dynamics through its influence on ET, a key factor that includes both soil evaporation and plant transpiration (Cowie *et al*., 2020). Earlier works have shown that weeds and crops are potent competitors for various growth factors such as water, nutrients, light and space, and therefore decrease crop productivity (Kaur *et al*., 2018). This competition directly impacts photosynthetic activity, while crop biomass production serves as a critical indicator of growth and yield potential (Duke & Patterson, 2018). Additionally, stress responses in plants include osmolytes accumulation (proline, sugars, amino acids), which help mitigate damage from water and oxidative stress (Jogawat, 2019).

This study aims to bridge the gap in understanding how parthenium infestation affects water budgeting and physiological responses in crops. We evaluated its impact on evapotranspiration, soil moisture, photosynthetic traits, and osmolytes accumulation in broccoli, lettuce, and peas. Measurements included gas exchange parameters using the LI-COR 6400 system, biomass production, and ETc. Additionally, 250 parthenium samples were analyzed for above- and below-ground relationships, including canopy cover, biomass, and root-shoot interactions to assess resource allocation. Normality analysis of these traits was conducted to ensure consistency and reliability in data interpretation. Additionally, a fuzzy logic model was employed to enhance water budgeting predictions by addressing variability and uncertainty in field conditions, particularly under the influence of invasive weed stress. This approach supports the development of an improved method for monitoring water dynamics in parthenium-infested agricultural systems.

## Material and Methods

**Survey study and sampling:** Parthenium is widely distributed across Pakistan, with its growth notably increasing during the rainy season. A survey was conducted from July to September 2023 in the Botanical Garden, University of the Punjab, Lahore (N 31° 30' 4.3236", E 74° 18' 5.4684"). Using a random sampling approach, 300 plants were collected, out of which 250 were identified as Parthenium, confirming its dominance in the area. The remaining 50 plants belonged to other species. This large sample size was selected to capture the morphological variability within the parthenium population and to reflect the infestation status in the study area. The data were used to analyze differences in morphological traits and to support further modeling and analysis.

**Parameters measured for parthenium analysis:** The frequency, density, relative frequency, and absolute frequency of parthenium were calculated using the following formulas:

$$\text{Frequency (\%)} = \frac{\text{Number of samples with parthenium}}{\text{Total number of samples}} \times 100$$

$$\text{Density (plants/m}^2\text{)} = \frac{\text{Total number of parthenium plants}}{\text{Total area}}$$

$$\text{Relative frequency} = \frac{\text{Number of samples with parthenium}}{\text{Total number of samples}}$$

$$\text{Absolute frequency} = \text{Number of samples with parthenium}$$

The results are presented in Table 1, which shows the calculated values for each above mentioned metric.

Morphological parameters including Shoot length (SL), number of branches (SL/NoB), number of leaves (SL/NoL), leaf width (SL/LW), leaf length (SL/LL), leaf area (SL/LA), leaf area index (SL/LAI), root length (SL/RL), number of root hairs (SL/NoRH), average root hair length (SL/ARHL), and plant length (SL/PL). Root length (RL), the number of root hairs (RL/NoRH) and the average root hair length (RL/ARHL), leaf width (LW), leaf length (LL), leaf area (LA), and leaf area index (LAI) were recorded. Plant weight measurements included fresh weights of small, medium, and large leaves (FWL-S, FWL-M, FWL-L), fresh weights of shoots (FWS), roots (FWR), and root hairs (FRRH), as well as their corresponding dry weights (DWL-S, DWL-M, DWL-L, DWS, DWR, DRRH). Canopy size or spread was recorded as canopy. Photosynthesis and chlorophyll content were assessed using SPAD values for small, medium, and large leaves (SPADL-S, SPADL-M, SPADL-L). Additional leaf measurements included the number of branches (NoB), number of leaves (NoL), number of root hairs (NoRH), average root hair length (ARHL), and plant length (PL). Overall ratios and indexes, such as leaf area index (LAI) and canopy size were also examined. Data normality was assessed, and results are provided in Supplementary Figs. S1–S8. Additional ratio analyses, including relationships between root parameters and above-ground traits, are provided in Supplementary Figs. S9–S10 to further support these findings.

**Derivation of canopy size as a single characteristic of water budget:** To quantify water consumption related to plant size, the canopy diameter (CD) was used as a representative feature of plant biomass and water use. The canopy diameter, as a single characteristic, was derived by measuring the width of the canopy at three distinct points during the growth stages. A model equation was developed to describe the relationship between canopy diameter and other growth parameters, such as plant height and leaf area.

**Experimental design for crop–weed interaction study:** The study was conducted using 10-liter plastic pots with a soil surface area of approximately 314 cm² and a water-holding capacity of 5.5 liters. Each pot was filled with a mixture of soil and organic matter in a 3:1 ratio. The soil was characterized as loamy with a pH of 7.2 and an organic matter content of 1.5%. Seeds of broccoli (*Brassica oleracea*), lettuce (*Lactuca sativa*), and peas (*Pisum sativum*) were sterilized using a 2% sodium hypochlorite solution for 10 minutes and then rinsed thoroughly with distilled water. The sterilized seeds were soaked overnight to enhance germination and then sown in their respective pots as shown (Fig. 1). by using randomized complete block design (RCBD). The experiment included three replicates for each treatment group to ensure consistency and statistical reliability. The treatment groups consisted of six different conditions: T1 (with parthenium at 14 DAS), T2 (without parthenium at 14 DAS), T3 (with parthenium at 25 DAS), T4 (without parthenium at 25 DAS), T5 (with parthenium at 40 DAS), and T6 (without parthenium at 40 DAS). These treatments allowed for the evaluation of parthenium's impact on water budgeting, photosynthetic activity, and biomass production in the crops at various growth stages.

Fig. 1. Experimental setup illustrating the cultivation of crops (broccoli, lettuce, and peas) with and without parthenium.

**Environmental condition:** The experiment was conducted in 2021 at the Botanical Garden of the University of the Punjab, Lahore, Pakistan (latitude: specifically, mapping master test site lies at geographical coordinates: latitude = 31° 49’ 81.50 “N; longitude = 74° 29’ 91.56 “E; and 214 meters above sea level. The daily temperature in November and December 2021 was between 12-19 degree Celsius, and humidity was between 20-70%. Levels of incoming solar radiation varies from 14 – 19 MJ/m² and wind speed varies from 2:4 – 3.1m/s. The climatic conditions for this experiment were taken from a wireless weather station (DIGITECH touch screen wireless weather station with USB PC Link XC0348) placed around the experimental site.

**Water budget components of crop and weed:** The inputs of water into the system were calculated based on precipitation, irrigation, and the contribution from atmospheric factors. The water retention within the system was measured through soil moisture content analysis, and the rate of water retention across different plant species was monitored continuously. Water outputs, including transpiration and evaporation, were determined through the calculation of crop evapotranspiration (ETc), which was derived from the ETo values using crop coefficients (Kc).

**Determination of ETo through penman–monteith equation:** ETo values were calculated from the Penman-Monteith equation, reflecting the ETo in a reference evapotranspiration scale. This experiment uses parameters such as temperature, relative humidity, solar radiation among others and wind speed to estimate the amount of water required by a crop at the optimum condition. For evaluation of the water requirements of the crops in the present study, ETo values were daily measured at 7-day interval during the overall length of the experiment. These calculated ETo values were then used to estimate the crop evapotranspiration (ETc) by using the crop coefficient multipliers to the ETo which gave the water loss in the experimental conditions (McNaughton & Jarvis, 1984). The equation is as follows:

$$\mathrm{ETo} = \frac{(0.408)\Delta(\mathrm{Rn\ G}) + \gamma\frac{900}{\mathrm{T}+273}\,\mathrm{u2}\,(\mathrm{es}-\mathrm{ea})}{\Delta + \gamma(1+0.34\mathrm{u2})}$$

**Crop evapotranspiration (ETc) determination:** Crop evapotranspiration (ETc) is calculated by multiplying the reference evapotranspiration (ETo) with the crop coefficient (Kc) provided by the FAO for peas, broccoli, and lettuce, as explained in Chapter 6 (ETc - Single crop coefficient) (Pereira *et al.*, 2021). The ETc for individual crops was determined using the specified formula:

$$\mathrm{ETc} = \mathrm{ETo} \times \mathrm{Kc}$$

Since no published Kc value exists for parthenium (weed), its Kc value was first estimated using the relationship between ETc and ETo. After determining the Kc for parthenium, the ETc of the combined system (parthenium and crops) was calculated using the same formula:

$$\mathrm{ETc\ combined} = \mathrm{ETo} \times (\mathrm{Kc}_{crop} + \mathrm{Kc}_{weed})$$

where:

- ETc combined = Combined evapotranspiration for the crop and parthenium
- ETo = Reference evapotranspiration (same for both the crop and parthenium)
- Kc crop = Crop coefficient for the specific crop (peas, broccoli, lettuce)
- Kc weed = Crop coefficient for parthenium (weed), calculated as described earlier

**Soil moisture content determination:** Soil moisture content was determined at three growth stages: 14, 25, and 40 days after sowing. Soil samples were collected from each pot, and the gravimetric method was used to determine the soil moisture content. The soil samples were weighed, oven-dried at 105°C for 24 hours and then re-weighed to determine the moisture content. The soil moisture content was calculated as the percentage of water lost during oven-drying relative to the initial soil weight (Schmugge *et al.*, 1980).

**Soil water loss (SWL) measurements:** Soil water loss was measured using a pot-based experiment, where pots were filled with a uniform soil mixture and planted with broccoli, lettuce, peas, and parthenium, both individually and in combination. An additional set of pots with bare soil (no plants) served as controls to account for soil evaporation. Pots were saturated with water, allowed to drain for 24 hours to achieve field capacity, and then weighed. Subsequent weighing was performed every 7 days at the same time of day to minimize temperature fluctuations. The weight difference between consecutive measurements was attributed to soil water loss, which included both plant transpiration and soil evaporation. By subtracting the water loss from the bare soil controls, the water loss due to plant transpiration was estimated for each treatment (Allen *et al.*, 1998).

**Treatment groups:** Table 1. Experimental treatment groups used for assessing the impact of parthenium on crop growth at different days after sowing

**Table 1.**

| | | |
|---|---|---|
| 1. | T1 with Parthenium | (P+ at 14 DAS) |
| 2. | T2 without Parthenium | (P- at 14 DAS) |
| 3. | T3 with Parthenium | (P+ at 25 DAS) |
| 4. | T4 without Parthenium | (P- at 25 DAS) |
| 5. | T5 with Parthenium | (P+ at 40 DAS) |
| 6. | T6 without Parthenium | (P- at 40 DAS) |

**DAS represents (days after sowing). 14 DAS:** Represents the seedling stage for lettuce and early root establishment. 25 DAS: Falls within the rapid vegetative growth phase. 40 DAS: Approaches the harvest stage/ mature vegetative phase, where water demand and resource competition are critical.

**Determination of chlorophyll and carotenoids:** Chlorophyll a, b, total chlorophyll, and carotenoids were quantified using acetone extraction. Fresh leaf samples (0.5 g) were homogenized in 80% acetone and centrifuged at 10,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 663, 645, and 470 nm using a spectrophotometer. The concentrations were calculated using standard formulas and expressed in mg/g fresh weight (Arnon, 1949).

**Determination of gas exchange parameters and osmolytes:** Photosynthetic parameters, including net photosynthesis, stomatal conductance, and transpiration rates, were measured with an LI-COR gas-exchange system (LI-6400; LICOR Biosciences, Lincoln, NE, USA). Measurements were taken on fully expanded leaves under natural light conditions between 9:00 AM and 11:00 AM to ensure consistency. Data were recorded across treatments and growth stages to assess physiological responses. Plant ethanol extracts were prepared to analyze non-enzymatic antioxidants and key osmolytes. For this, 50 mg of dry plant material was homogenized in 10 mL of 80% ethanol and filtered through Whatman No. 41 filter paper. The residue was re-extracted with ethanol, and both extracts were combined to a final volume of 20 mL. Sugars (Dubois *et al.*, 1956) and amino acids (Blackburn, 1968) were quantified from the extracts. Free proline content (Bates *et al.*, 1973) was measured using a standard curve at 520 nm absorbance and expressed as µmol $g^{-1}$ FW.

**Statistical Analysis**

Statistical analysis was conducted using SPSS software to determine the significance of differences among treatments. Graphical representation of data, including bar charts and scatter plots, was performed using Origin Pro software, while line graphs were generated using Microsoft Excel. Correlation analysis and heatmap generation for ETo and ETc values of Parthenium and crops were carried out using R software to visualize relationships and patterns effectively.

**Results**

**Plant population metrics for parthenium infestation in the botanical garden:** Below Table 2 provides an overview of the key plant population metrics for parthenium infestation, including frequency, density, relative frequency, and absolute frequency, observed in the botanical garden. These metrics offer a comprehensive view of parthenium's spread and density in the study area. The results indicate a high frequency (83.33%) of Parthenium in the botanical garden, with an absolute frequency of 250 plants. The density of parthenium was relatively low at 0.2 plants/m², suggesting widespread distribution in the study area.

**Table 2. Overview of plant population metrics.**

| Sr. No. | Metrics | Value | Units |
|---|---|---|---|
| 1. | Frequency | 83.33 | % |
| 2. | Density | 0.2 | Plants/$m^2$ |
| 3. | Relative frequency | 0.8333 | - |
| 4. | Absolute frequency | 250 | Plants |

**Relationship of Canopy/PL ratio with both above-ground and below-ground parameters:** The Canopy/PL ratio exhibited strong correlations with both above-ground and below-ground parameters in parthenium, highlighting its critical role in overall plant architecture as shown (Fig. 2). Among above-ground traits, the Canopy/PL ratio showed a high correlation with SL/NoB ($R^2 = 0.9104$) and SL/NoL ($R^2 = 0.9146$), suggesting that canopy expansion is closely tied to shoot branching and leaf production. The SL/PL ratio ($R^2 = 0.9029$) further reinforced the interdependence between canopy development and total plant growth. Additionally, SL/LW ($R^2 = 0.8482$) and SL/LL ($R^2 = 0.9072$) confirmed the contributions of leaf width and length to canopy formation, while SL/LA ($R^2 = 0.9054$) and SL/LAI ($R^2 = 0.9996$) underscored the importance of leaf area and leaf area index in determining canopy coverage. Below-ground parameters also showed strong associations with the Canopy/PL ratio, emphasizing the influence of root traits on plant structure. The correlation between Canopy/PL and RL/NoRH ($R^2 = 0.901$) suggested that root length relative to the number of root hairs plays a crucial role in canopy expansion. A stronger correlation with RL/ARHL ($R^2 = 0.9796$) indicated that average root hair length significantly impacts plant stature and canopy spread.

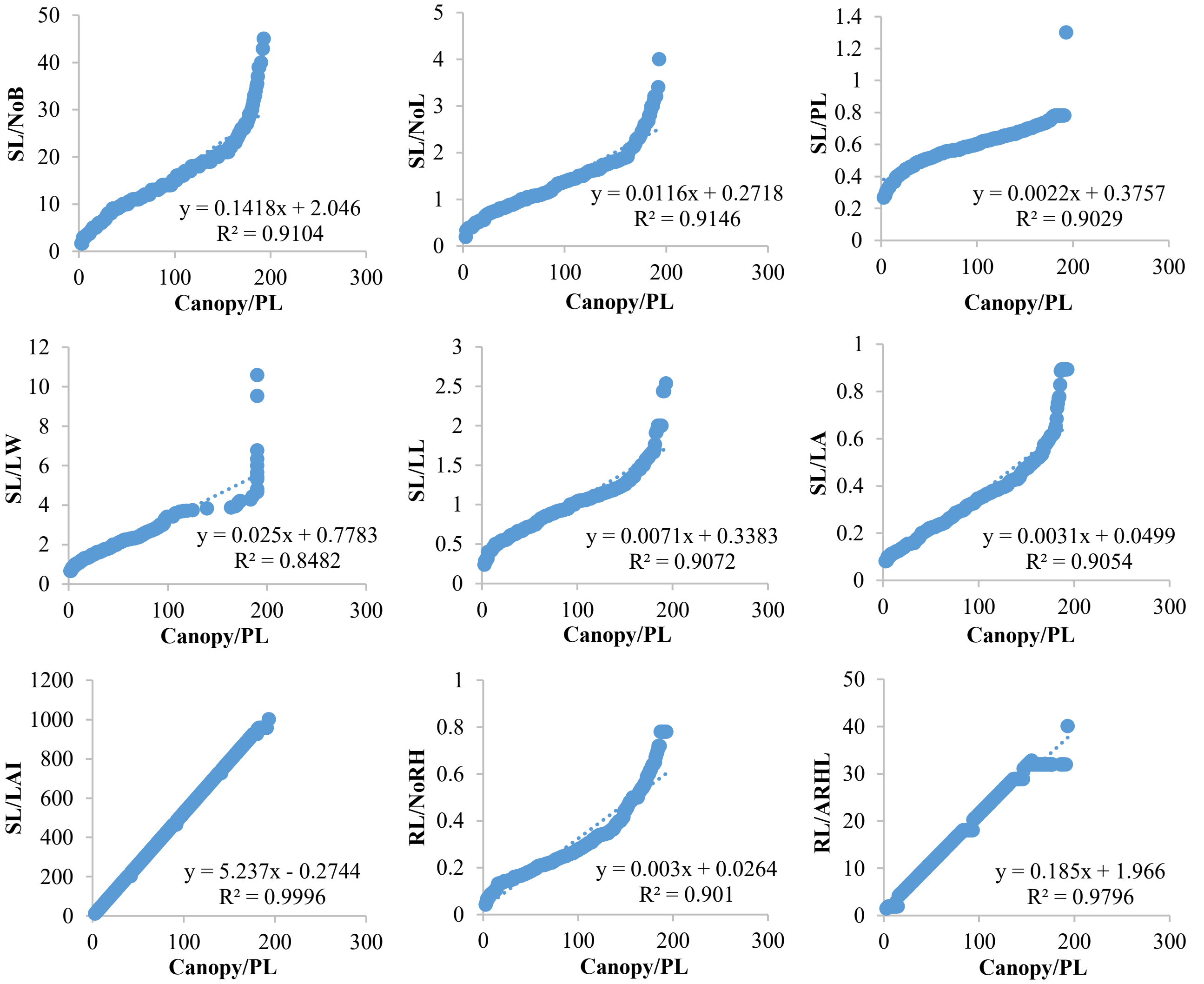


Fig. 2. Relationship between Canopy/PL to above and below ground parameter ratios (SL/NoB, SL/NoL, SL/PL, SL/LW, SL/LL, SL/LA, SL/LAI, RL/NoRH, RL/ARHL) in parthenium samples.

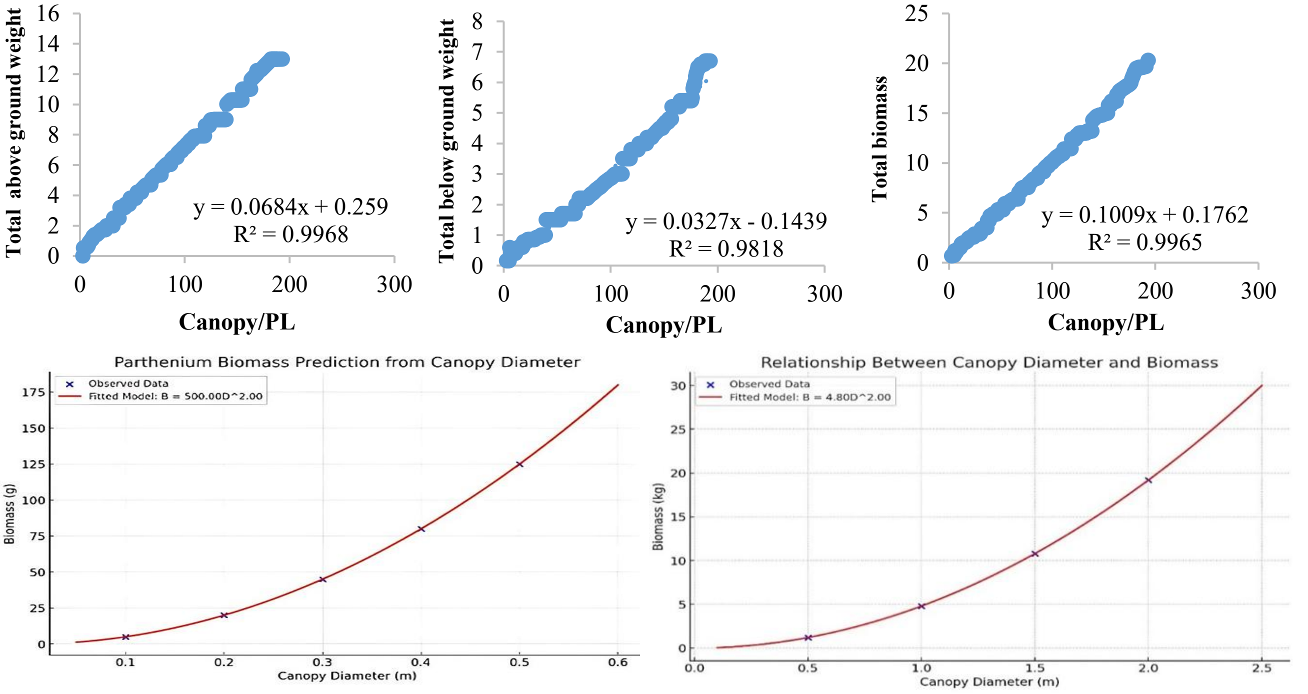


Fig. 3. Relationship between Canopy/PL and biomass production in parthenium samples and biomass prediction from canopy diameter and relationship between canopy diameter and biomass.

**Table 3. Impact of Parthenium on plant height, root length, plant fresh weight, plant dry weight, root dry weight of selected crops. (mean ± SD).**

| Crop | Treatment | Plant height (cm) | Root length (cm) | Plant fresh weight (g) | Plant dry weight (g) | Root fresh weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|---|---|
| Broccoli | P + at 14 DAS | 8.4 ± 0.9$^{e}$ | 3.1 ± 0.6$^{bc}$ | 5.0 ± 0.5$^{e}$ | 1.1 ± 0.2$^{cd}$ | 1.2 ± 0.3$^{c}$ | 0.6 ± 0.1$^{b}$ |
| | P - at 14 DAS | 9.8 ± 0.8$^{e}$ | 3.4 ± 0.7$^{bc}$ | 5.2 ± 0.6$^{e}$ | 1.2 ± 0.2$^{cd}$ | 1.5 ± 0.4$^{b}$ | 0.67 ± 0.1$^{b}$ |
| | P + at 25 DAS | 18.1 ± 1.5$^{c}$ | 4.1 ± 1.0$^{b}$ | 7.6 ± 1.2$^{bc}$ | 2.1 ± 0.3$^{b}$ | 1.8 ± 0.5$^{ab}$ | 0.73 ± 0.3$^{ab}$ |
| | P - at 25 DAS | 22.3 ± 1.7$^{b}$ | 5.1 ± 1.2$^{ab}$ | 8.1 ± 1.5$^{b}$ | 2.4± 0.4$^{b}$ | 2.0 ± 0.6$^{a}$ | 0.8 ± 0.4$^{a}$ |
| | P + at 40 DAS | 30.3 ± 2.5$^{b}$ | 5.2 ± 1.6$^{ab}$ | 10.6 ± 2.0$^{ab}$ | 3.2± 0.5$^{a}$ | 2.0 ± 0.8$^{a}$ | 0.78 ± 0.5$^{a}$ |
| | P - at 40 DAS | 34.8 ± 2.8$^{a}$ | 6.1 ± 1.8$^{a}$ | 11.4 ± 2.3$^{a}$ | 3.4± 0.6$^{a}$ | 2.3 ± 1.0$^{a}$ | 0.88 ± 0.4$^{a}$ |
| Lettuce | P + at 14 DAS | 5.2 ± 0.6$^{fg}$ | 1.5 ± 0.4$^{cd}$ | 2.8 ± 0.3$^{fg}$ | 0.571 ± 0.1$^{d}$ | 0.92 ± 0.3$^{cd}$ | 0.52 ± 0.1$^{c}$ |
| | P - at 14 DAS | 6.4 ± 0.5$^{f}$ | 1.7 ± 0.5$^{cd}$ | 3.2 ± 0.3$^{f}$ | 0.78 ± 0.1$^{d}$ | 1.0 ± 0.2$^{c}$ | 0.55 ± 0.2$^{bc}$ |
| | P + at 25 DAS | 12.9 ± 1.0$^{d}$ | 2.1 ± 0.7$^{c}$ | 5.4 ± 0.5$^{de}$ | 1.2± 0.2$^{d}$ | 1.2 ± 0.3$^{c}$ | 0.6 ± 0.2$^{b}$ |
| | P - at 25 DAS | 14.4 ± 1.2$^{d}$ | 2.6 ± 0.8$^{c}$ | 6.2 ± 0.6$^{d}$ | 1.6 ± 0.2$^{c}$ | 1.5 ± 0.3$^{b}$ | 0.69 ± 0.3$^{b}$ |
| | P + at 40 DAS | 21.1 ± 1.6$^{bc}$ | 2.8 ± 1.1$^{c}$ | 6.7 ± 0.8$^{cd}$ | 1.8 ± 0.3$^{c}$ | 1.33 ± 0.4$^{bc}$ | 0.62 ± 0.2$^{b}$ |
| | P - at 40 DAS | 23.7 ± 1.8$^{b}$ | 3.3 ± 1.3$^{bc}$ | 7.2 ± 1.0$^{c}$ | 2.1 ± 0.3$^{b}$ | 1.8 ± 0.5$^{ab}$ | 0.76 ± 0.2$^{ab}$ |
| Peas | P + at 14 DAS | 7.4 ± 0.8$^{ef}$ | 2.2 ± 0.5$^{c}$ | 4.1 ± 0.4$^{ef}$ | 1.0 ± 0.1$^{cd}$ | 1 ± 0.2$^{c}$ | 0.55 ± 0.3$^{bc}$ |
| | P - at 14 DAS | 8.6 ± 0.7$^{e}$ | 2.4 ± 0.6$^{c}$ | 5.0 ± 0.5$^{de}$ | 1.3 ± 0.5$^{cd}$ | 1.2 ± 0.2$^{c}$ | 0.68 ± 0.4$^{b}$ |
| | P + at 25 DAS | 16.2 ± 1.2$^{cd}$ | 3.1 ± 0.8$^{bc}$ | 6.5 ± 0.7$^{d}$ | 1.7 ± 0.2$^{c}$ | 1.3 ± 0.3$^{bc}$ | 0.69 ± 0.2$^{b}$ |
| | P - at 25 DAS | 18.7 ± 1.3$^{c}$ | 3.6 ± 0.9$^{b}$ | 7.2 ± 0.8$^{c}$ | 2.1 ± 0.2$^{b}$ | 1.6 ± 0.4$^{b}$ | 0.72 ± 0.1$^{ab}$ |
| | P + at 40 DAS | 30.3 ± 1.9$^{b}$ | 5.5 ± 1.2$^{a}$ | 7.8 ± 1.0$^{bc}$ | 2.4 ± 0.6$^{b}$ | 1.5 ± 0.5$^{b}$ | 0.73 ± 0.3$^{ab}$ |
| | P - at 40 DAS | 35.8 ± 2.2$^{a}$ | 5.9 ± 1.4$^{a}$ | 8.1 ± 1.2$^{b}$ | 2.7 ± 0.4$^{ab}$ | 2.0 ± 0.6$^{a}$ | 0.88 ± 0.2$^{a}$ |

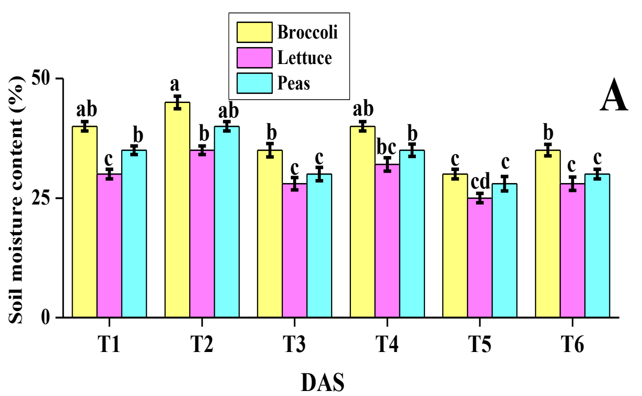


Fig. 4. Depicts the soil moisture content percentage **(A)** in the soil of broccoli, lettuce, and peas with and without parthenium. The treatments are as follows: T1 (P+ at 14 DAS), T2 (P- at 14 DAS), T3 (P+ at 25 DAS), T4 (P- at 25 DAS), T5 (P+ at 40 DAS), and T6 (P- at 40 DAS).

**Relationship between canopy and plant length (PL) to biomass of parthenium samples and biomass prediction:** The Canopy/PL ratio showed a strong correlation with biomass accumulation in parthenium. Above-ground biomass ($R^2$ = 0.9968) and below-ground biomass ($R^2$ = 0.9818) were both closely linked to canopy expansion and plant height. The total biomass correlation ($R^2$ = 0.9965) confirmed the interdependence between structural growth and biomass allocation (Fig. 4). Biomass prediction followed a quadratic pattern, with models $B = 500.00D^2$ and $B = 4.80D^2$ accurately describing the biomass-canopy relationship as shown (Fig. 3). Differences in coefficients were due to unit variations. While useful for biomass estimation, model reliability is limited to the measured range and environmental conditions.

**Impact of Parthenium on different vegetative parameters of crops:** The presence of parthenium had a consistently negative impact on the growth and biomass of broccoli, lettuce, and peas across all sampling stages (Table 3). In broccoli, plants grown with parthenium (T1, T3, T5) exhibited reduced plant height, root length, and fresh weight compared to those grown without parthenium (T2, T4, T6). For example, at 25 DAS, broccoli plants with parthenium (T3) had a height of 20.1 cm, significantly lower than the 24.3 cm in the absence of parthenium (T4). Similar trends were observed for root length, fresh, and dry weights. At 40 DAS, the disparity became more pronounced, indicating that prolonged exposure to parthenium exacerbates its negative effects on plant growth. Lettuce and peas followed a similar pattern. Lettuce grown with parthenium at 25 DAS (T3) showed a height of 12.8 cm, compared to 14.4 cm in the absence of parthenium (T4). Likewise, peas at 40 DAS with parthenium (T5) were 29.3 cm tall, compared to 34.8 cm without parthenium (T6).

**Impact of parthenium on soil moisture content:** The results in Fig. 4. indicate that the presence of parthenium significantly reduced soil moisture content across all crops. For broccoli, soil moisture content dropped by 11.11% at 14 DAS, 12.5% at 25 DAS, and 14.29% at 40 DAS due to the presence of parthenium. In lettuce, soil moisture content decreased by 14.29% at 14 DAS, 12.5% at 25 DAS, and 10.71% at 40 DAS when parthenium was present. For peas, soil moisture content fell by 12.5% at 14 DAS, 14.29% at 25 DAS, and 6.67% at 40 DAS. Overall, the presence of parthenium led to a significant reduction in soil moisture content, exacerbating water stress and negatively affecting the growth and development of the crops.

**Impact of parthenium on chlorophyll and carotenoids content:** The chlorophyll *a* content in plants showed a significant reduction due to the presence of *parthenium* (P+). At 14 DAS, chlorophyll *a* levels were lower in P+ plants (broccoli: 0.82 mg $g^{-1}$ FW, lettuce: 0.75 mg $g^{-1}$ FW, peas: 0.75 mg $g^{-1}$ FW) compared to P- plants (broccoli: 0.91 mg $g^{-1}$ FW, lettuce: 0.82 mg $g^{-1}$ FW, peas: 0.88 mg $g^{-1}$ FW). This trend continued at 25 DAS, and by 40 DAS, the P+ plants exhibited further decline in chlorophyll *a* (broccoli: 1.52 mg $g^{-1}$ FW, lettuce: 1.25 mg $g^{-1}$ FW, peas: 1.32 mg $g^{-1}$ FW) compared to P- plants (broccoli: 1.65 mg $g^{-1}$ FW, lettuce: 1.4 mg $g^{-1}$ FW, peas: 1.4 mg $g^{-1}$ FW), likely due to water loss and senescence caused by *parthenium* interference. Chlorophyll *b* and total chlorophyll content showed a significant reduction in *parthenium* (P+) treatments compared to control (P-) plants. This reduction was particularly noticeable at 14 DAS and 40

DAS, where plants grown with *parthenium* had lower chlorophyll *b* levels, indicating a negative impact of *parthenium* on the photosynthetic capacity of broccoli, lettuce, and peas. The highest decline was observed in broccoli and lettuce at 40 DAS, suggesting increased stress and potential onset of senescence in the presence of *parthenium*. Carotenoid content (mg $g^{-1}$ FW) displayed a similar trend to chlorophyll, with higher values in *parthenium*-free (P-) plants compared to those exposed to *parthenium* (P+). For instance, at 14 DAS, carotenoid levels in P- treatments were 0.23 mg $g^{-1}$ FW in broccoli, 0.21 mg $g^{-1}$ FW in lettuce, and 0.24 mg $g^{-1}$ FW in peas. This trend continued at 25 DAS, with significantly higher carotenoid content in P- plants. However, by 40 DAS, the difference in carotenoid content between P+ and P- treatments was not significant across all crops, suggesting that *parthenium* did not substantially affect carotenoid levels at later growth stages (Fig. 5).

**Impact of parthenium on gas exchange parameters:** The present research work evaluated the effects of *P. hysterophorus* on the broccoli, lettuce, and peas at different growth stages using qualitative and quantitative characteristics of photosynthetic activity determined using the readings from the IRGA parameters as (Fig. 6). Carbon assimilation and plant growth were quantified by net photosynthesis, stomatal conductance, transpiration and $CO_2$ uptake rates. The highest net photosynthesis (µmol $m^{-2}s^{-1}$) values were observed in the T4 (P- at 25 DAS) treatment for all crops: broccoli 8.3 µmol $m^{-2}s^{-1}$, lettuce 6.2 µmol $m^{-2}s^{-1}$ and peas 7.2 µmol $m^{-2}s^{-1}$. These values were significantly higher compared to T1 (P+ at 14 DAS), which showed the lowest photosynthesis: broccoli was 4.8 µmol $m^{-2}s^{-1}$ followed by lettuce (3 µmol $m^{-2}s^{-1}$) and peas (4 µmol $m^{-2}s^{-1}$). Stomatal conductance followed a similar pattern, with higher values in T4 (P- at 25 DAS): broccoli lost more water vapor at a rate of 0.32 µmol $m^{-2}s^{-1}$, followed by lettuce at 0.22 µmol $m^{-2}s^{-1}$ and peas at 0.27 µmol $m^{-2}s^{-1}$ overlaying better water vapor loss efficiency with no growth of parthenium. Also at 25 DAS, stomatal conductance was higher in T4 and hence the higher transpiration rates were also recorded in broccoli (4.0 µmol $m^{-2}s^{-1}$), lettuce (3.0 µmol $m^{-2}s^{-1}$) and peas (3.5 µmol $m^{-2}s^{-1}$). On the other hand T1 (P+ at 14 DAS) had low stomatal conductance, transpiration and $CO_2$ assimilation being under stress due to parthenium competition. In general, the findings indicate that P+ had a detrimental effect on the physiological characteristics of growing crops most especially at young stages (e.g., T1 at 14DAS).

**Impact of parthenium on osmolytes:** At 14 DAS, reducing sugar content was higher in parthenium-treated plants (T1) compared to control (T2), with the highest value observed in lettuce (0.6 mg $g^{-1}$ FW). Non-reducing sugars followed a similar trend, with lettuce accumulating the most (11.5 mg $g^{-1}$ FW). Proline and free amino acid levels were also elevated in T1, with lettuce showing the highest accumulation (0.95 µmol $g^{-1}$ FW and 2.3 mg $g^{-1}$ FW, respectively). At 25 DAS, osmolytes accumulation increased further in T3 compared to controls (T4). Reducing sugars were highest in lettuce (1.2 mg $g^{-1}$ FW), followed by peas and broccoli. Non-reducing sugars peaked in lettuce (16 mg $g^{-1}$ FW). Proline and free amino acids also increased in T3, with lettuce maintaining the highest levels (1.2 µmol $g^{-1}$ FW and 3 mg $g^{-1}$ FW, respectively). Control plants (T4) showed lower values across all osmolytes. At 40 DAS, osmolytes accumulation was at its highest in parthenium-treated plants (T5). Lettuce again showed the greatest increase in reducing sugars (1.5 mg $g^{-1}$ FW), non-reducing sugars (20 mg $g^{-1}$ FW), proline (1.75 µmol $g^{-1}$ FW), and free amino acids (3.7 mg $g^{-1}$ FW). In contrast, control plants (T6) maintained lower osmolytes levels, indicating a clear stress-induced response in parthenium-treated crops. Overall, lettuce exhibited the highest osmolytes accumulation across all time points, followed by peas and broccoli as (Fig. 7).

**Effect of parthenium on transpiration and water loss in crop combinations:** The transpiration loss was highest when parthenium was grown alone, with values of 4.0 µmol $m^{-2}$ $s^{-1}$ at day 7 and gradually decreasing over time. This suggests that parthenium, without competition, maintained higher transpiration rates throughout the study period. However, when grown in combination with crops such as broccoli, lettuce, and peas, transpiration loss was consistently lower from parhtenium. This reduction in transpiration loss indicates that the competition for water and nutrients between parthenium and the crops led to a decrease in water loss through transpiration. By day 56, the transpiration loss was lowest in lettuce + parthenium (1.9 µmol $m^{-2}$ $s^{-1}$), followed by peas + parthenium (2.0 µmol $m^{-2}$ $s^{-1}$) and broccoli + parthenium (2.1 µmol $m^{-2}$ $s^{-1}$), demonstrating the competitive effects on transpiration efficiency when crops and parthenium were grown together. As shown (Fig. 8). Water loss from broccoli and parthenium was higher compared to the other crops, lettuce and peas. This indicates that the presence of parthenium, when grown in combination with crops, resulted in increased water loss due to heightened competition for water and nutrients.

**Impact of parthenium on crop evapotranspiration (ETc):** The data on crop evapotranspiration (ETc) clearly show the impact of parthenium on water loss, with significantly higher ETc values when grown in combination with crops compared to when grown alone Fig. 9. For lettuce grown alone, ETc ranged from 1.31 to 3.11 µmol $m^{-2}$ $s^{-1}$, while when grown with parthenium, ETc increased significantly, ranging from 3.31 to 7.88 µmol $m^{-2}$ $s^{-1}$, highlighting the effect of parthenium in increasing water loss through evapotranspiration. The ETc for broccoli grown alone ranged from 1.31 to 3.27 µmol $m^{-2}$ $s^{-1}$. However, when broccoli was grown with parthenium, the ETc values were much higher, ranging from 3.31 to 8.08 µmol $m^{-2}$ $s^{-1}$. This increase demonstrates the heightened competition for water between parthenium and the crop. For peas, the ETc values ranged from 0.76 to 3.36 µmol $m^{-2}$ $s^{-1}$ when grown alone. In combination with parthenium, the ETc increased from 2.76 to 7.36 µmol $m^{-2}$ $s^{-1}$, again showing the effect of parthenium on elevating evapotranspiration. Parthenium alone had an ETc range of 2.00 to 5.20 µmol $m^{-2}$ $s^{-1}$, which is considerably higher than that of the crops alone, further emphasizing the water demands of parthenium in comparison to the crops. Overall, the results clearly demonstrate that the presence of parthenium in combination with crops (broccoli, lettuce, or peas) increases the ETc, signifying higher water loss due to increased competition for water resources. The ETc values were consistently higher when crops were grown with parthenium, underlining its significant effect on evapotranspiration. In addition, both ETc and ETo showed a significant decrease from November to December, corresponding to a drop in temperature. This reduction in temperature led to lower evapotranspiration rates, highlighting the seasonal variation in water loss associated with temperature changes.

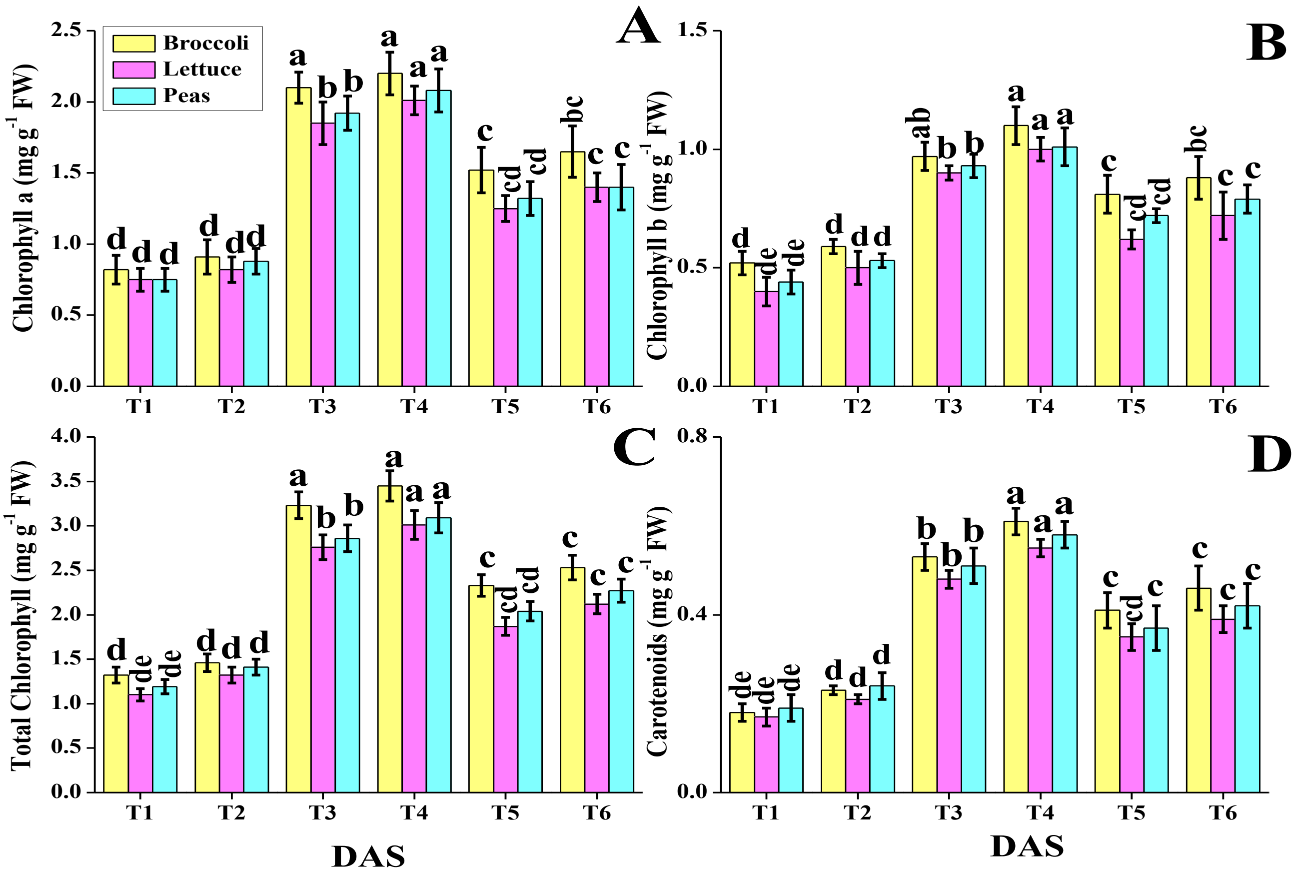


Fig. 5. Depicts the chlorophyll a **(A)** chlorophyll b **(B)** total chlorophyll content **(C)**, and carotenoids **(D)** of broccoli, lettuce, and peas with and without parthenium. The treatments are as follows: T1 (P+ at 14 DAS), T2 (P- at 14 DAS), T3 (P+ at 25 DAS), T4 (P- at 25 DAS), T5 (P+ at 40 DAS), and T6 (P- at 40 DAS).

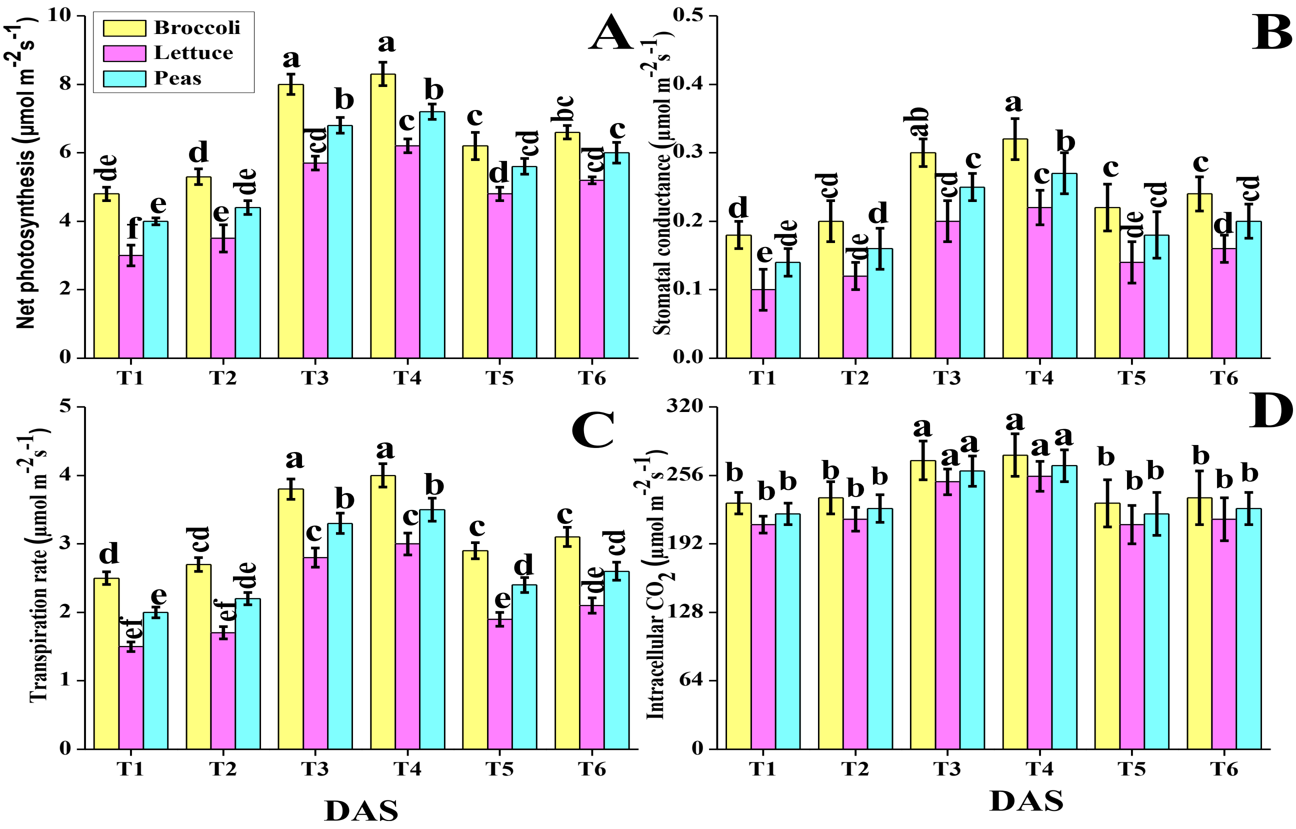


Fig. 6. Depicts the net photosynthesis rate (A), stomatal conductance (B), transpiration rate (C), intracellular $CO_2$ of broccoli, lettuce (D), and peas grown with and without parthenium. The treatments are as follows: T1 (P+ at 14 DAS), T2 (P- at 14 DAS), T3 (P+ at 25 DAS), T4 (P- at 25 DAS), T5 (P+ at 40 DAS), and T6 (P- at 40 DAS).

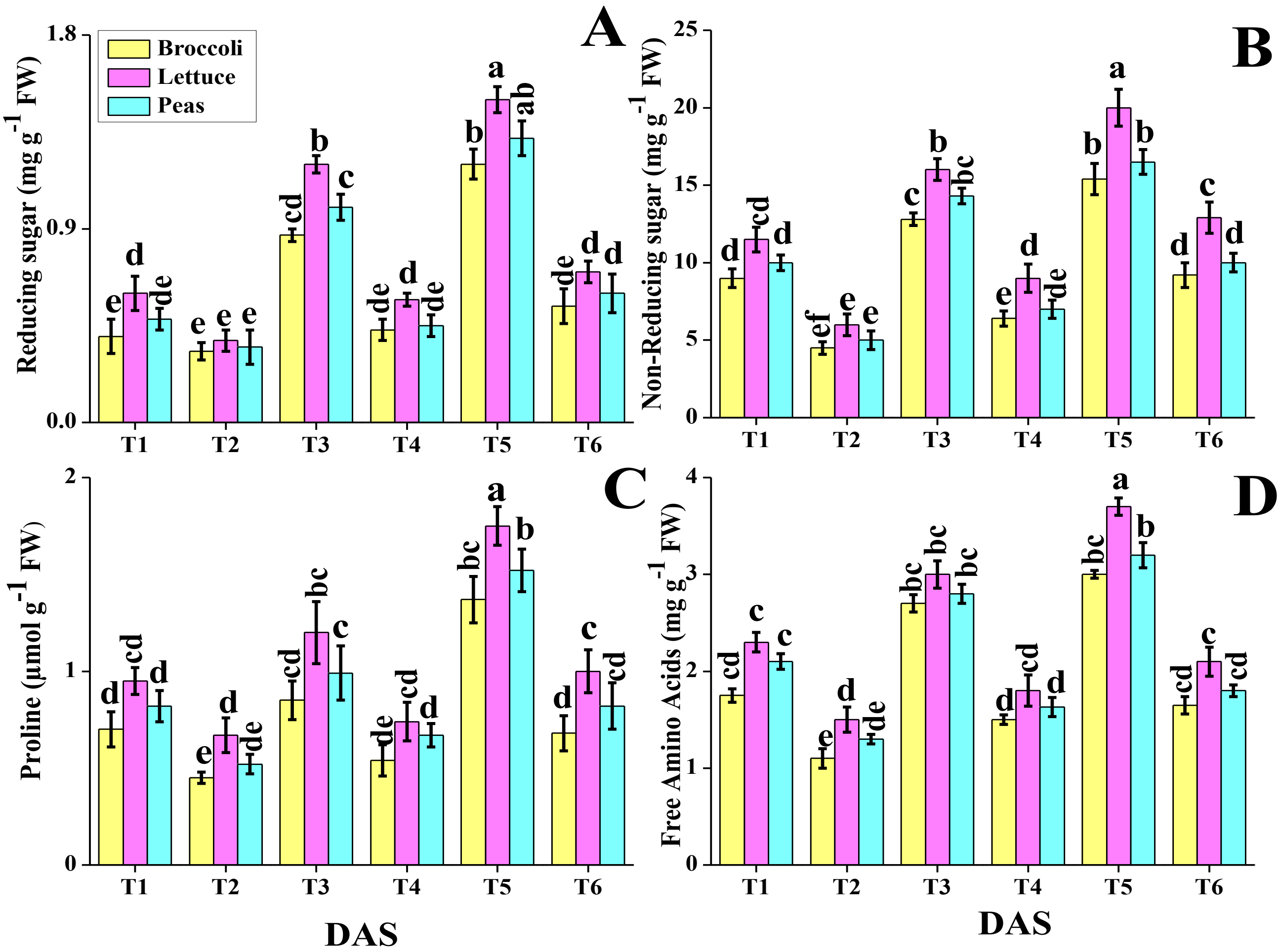


Fig. 7. Depicts the osmolytes including reducing sugar (A), non-reducing sugar (B), proline (C), and free amino acids (D). The treatments are as follows: T1 (P+ at 14 DAS), T2 (P- at 14 DAS), T3 (P+ at 25 DAS), T4 (P- at 25 DAS), T5 (P+ at 40 DAS), and T6 (P- at 40 DAS).

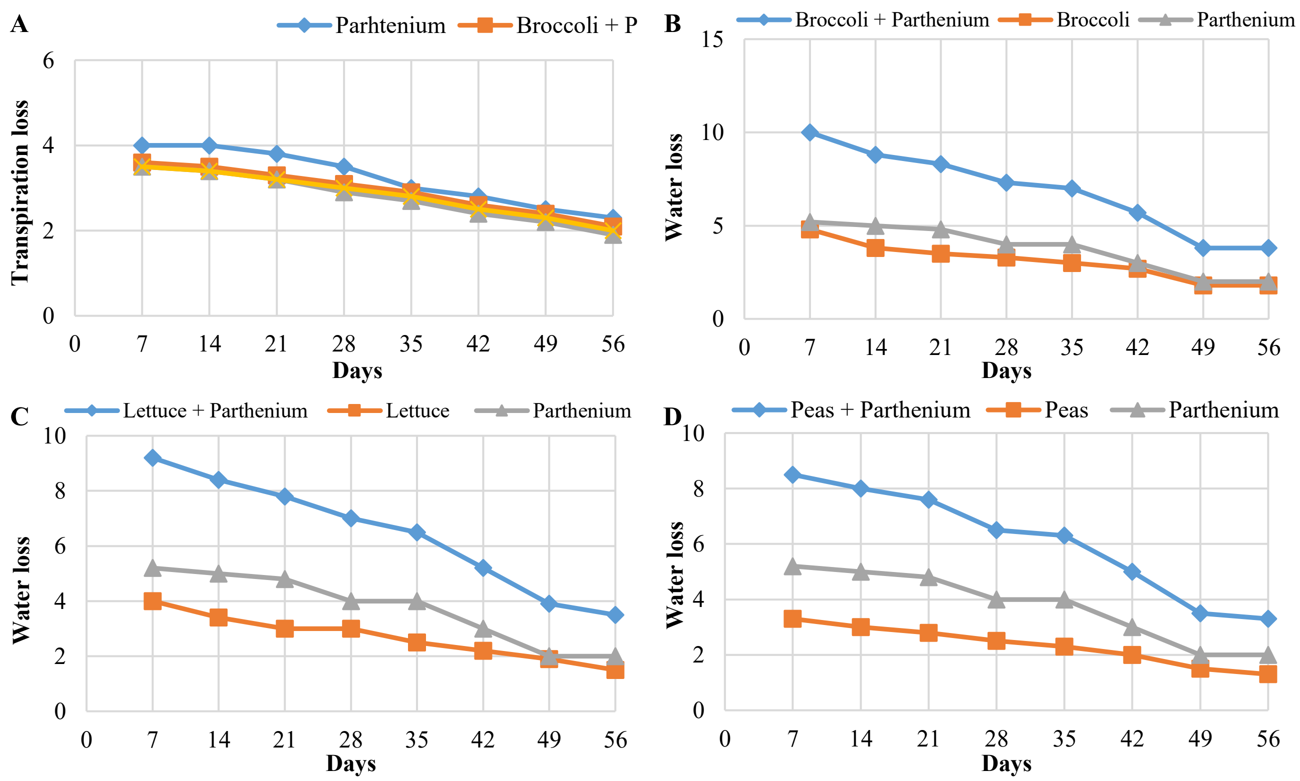


Fig. 8. Transpiration and water loss measurements in parthenium plants (A) transpiration loss in parthenium plants grown in combination with crops and alone (B) water loss from parthenium plants with and without broccoli (C) water loss from parthenium plants with and without lettuce (D) Water loss from parthenium plants with and without peas. All measurements were taken after 7 days of growth.

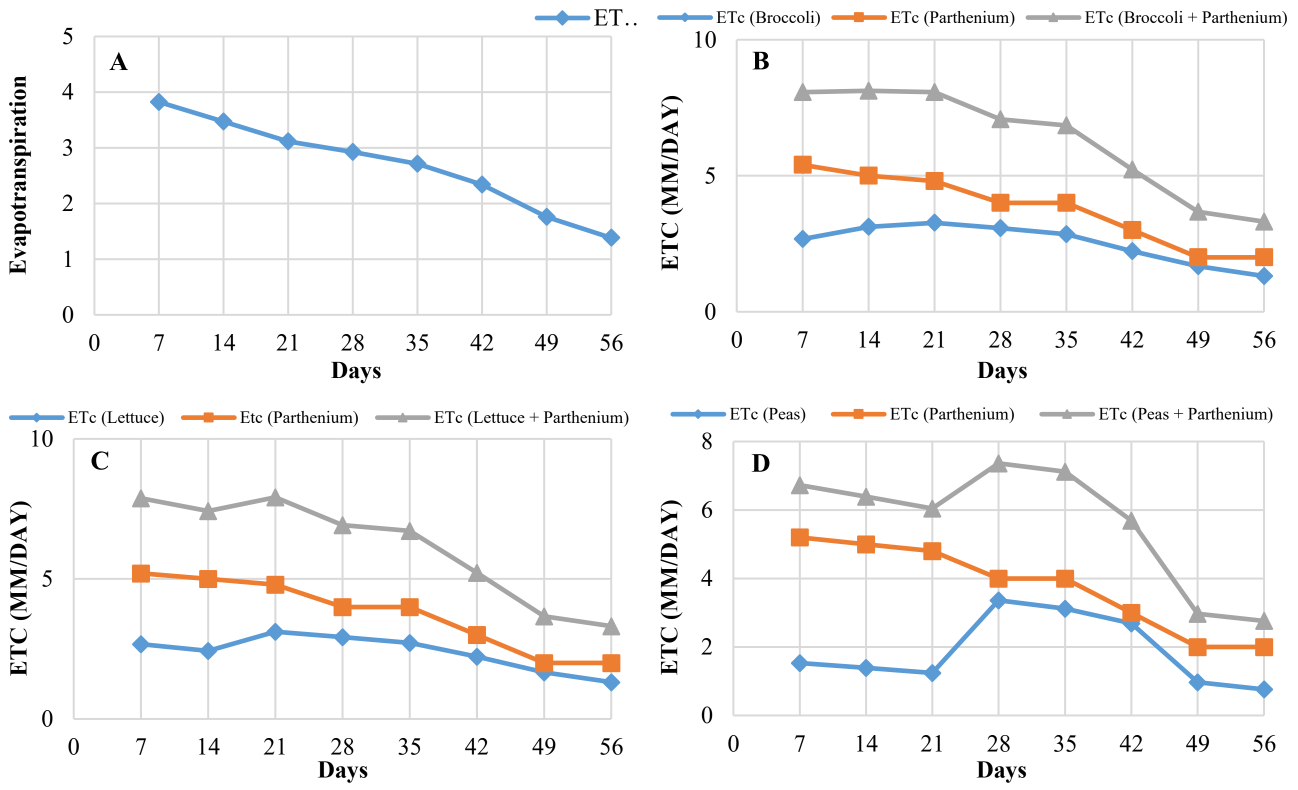


Fig. 9. Evapotranspiration measurements of parthenium and crops (A) reference evapotranspiration (same for both the crop and parthenium) (B) ETc of broccoli with and without parthenium (C) ETc of lettuce with and without parthenium (D) ETc of peas with and without parthenium. All measurements were taken after 7 days of growth.

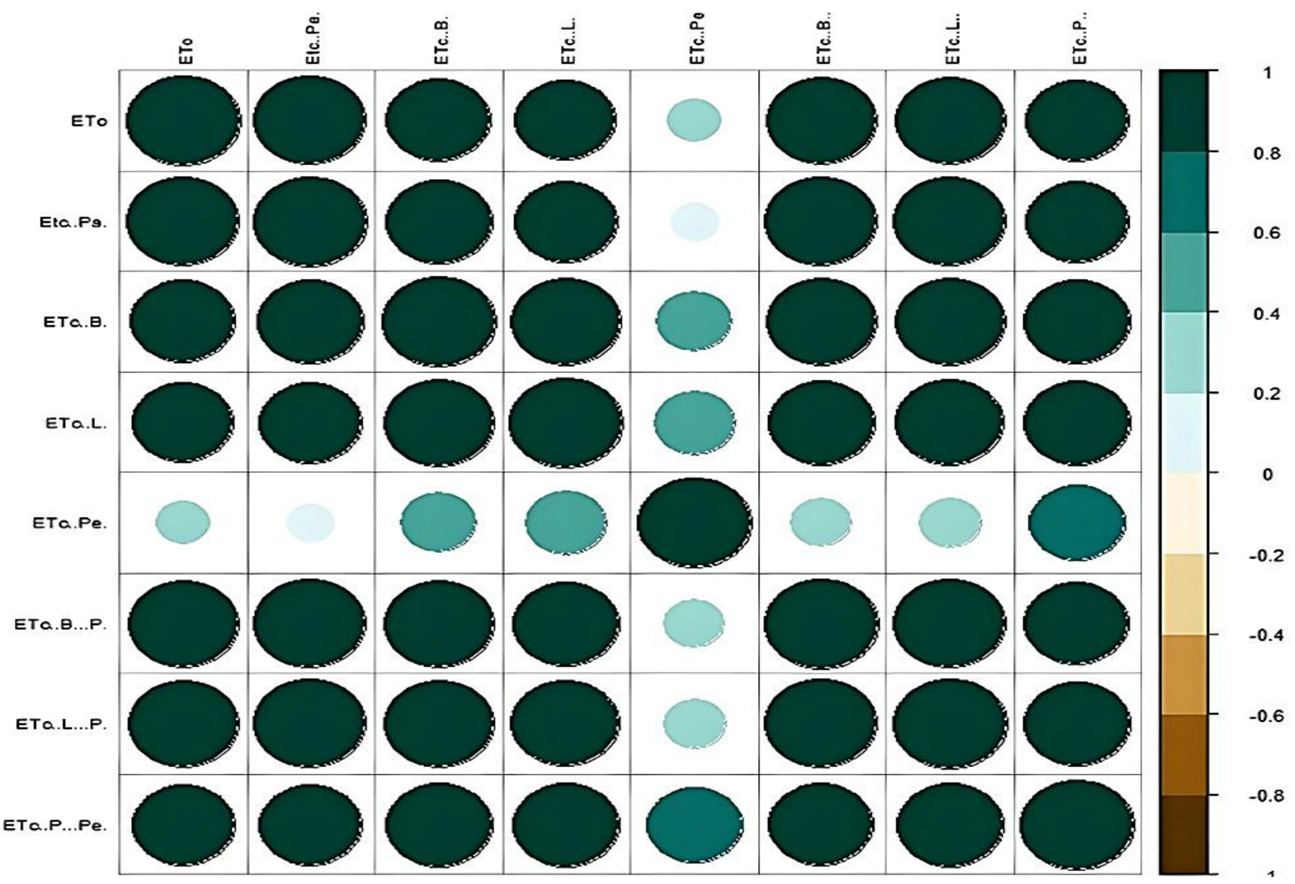


Fig. 10. Pearson Correlation of ETc value of parthenium and crops. Abbreviations used ETo referenced evapotranspiration, ETc. Pa. (evapotranspiration of parthenium), ETc. B (evapotranspiration of broccoli), ETc. L (evapotranspiration of lettuce), Etc. Pe. (Evapotranspiration of peas), ETc. B.P (combine evapotranspiration of broccoli and parthenium), ETc. L. P (combine evapotranspiration of lettuce and parthenium), ETc. P. Pa (combine evapotranspiration of peas and parthenium).

**Correlation analysis and heat map representation of ETo and ETc values of parthenium and crops:** The coefficient of 0.8-1 for broccoli, lettuce and parthenium, means plants relations to water use are positive and homogeneous during the experimental period (Fig. 10). The evapotranspiration (ETc) losses from the crops are highly positively related, while the water used by the crops behaves similarly under the same circumstances. ETc of peas with parthenium was significantly lower than other crops so it prevails a lighter color on the graph representing lower ETc value showing that it consumes less water. This implies that Peas have a low water loss potential than the peas grown with the combination of parthenium because of transpiration, faster growth or water use rate. The higher ETc value of parthenium, although represented here in a darker shade which may have an added disadvantage of competing with the crops for water as our study depicted.

**Fuzzy logic model outcome:** The fuzzy logic model effectively categorized water availability, competition intensity, and relative water allocation, demonstrating its ability to handle uncertainty in resource dynamics (Fig. S11). Water availability varied across low (≤300 mm), medium (200–400 mm), and high (≥500 mm) irrigation levels, influencing competition intensity and water allocation. The model predicted that precipitation increases total water input and retention, enhancing water savings, while its absence reduces water availability and intensifies competition. In a co-cultivated system, parthenium dominated water use (75%), leaving lettuce with 25%, yet a 1.0 L/day irrigation maintained a surplus. Precipitation further ensured adequate water levels, preventing stress during peak demand. Adjusted irrigation balanced water use, limiting excessive parthenium uptake and optimizing crop water availability, highlighting the role of strategic irrigation in mitigating competitive losses.

**Discussion**

Water is an essential component for plant growth, but its low availability in the soil causes great yield loss. One of the major reasons for limited water resources is the uncontrolled growth of weeds such as parthenium (Soltani *et al*., 2017). *Parthenium hysterophorus*, initially observed in East Punjab during the late 1990s, has rapidly spread across Pakistan from the northern to southern regions due to its aggressive growth and lack of control measures. The present study provides comprehensive insights into the morphological traits and ecological dominance of Parthenium. A key objective of this research was to investigate how parthenium infestation influences soil water balance by assessing its role in water uptake and evapotranspiration losses. Normality analysis of parthenium ratios confirmed a near-normal distribution among samples, validating the use of parametric approaches in evaluating plant responses. *Parthenium hysterophorus* competes aggressively with crops for water and nutrients, particularly under arid and water-limited conditions. Its allelopathic properties further suppress crop germination and interfere with normal physiological processes. In this study, the presence of parthenium significantly reduced the photosynthetic rate and biomass production of broccoli, lettuce, and peas, confirming its detrimental impact on crop growth. These findings underscore the competitive dominance of parthenium and the urgent need for effective weed management strategies. Similar reductions in yield have been reported in previous studies, such as a 50% yield loss in maize and 52% in soybean due to intense weed-crop competition (Soltani *et al*., 2016; Soltani *et al*., 2022).

A study in India showed a great loss of about 36% in peanuts and about 31% in soybeans, less than peanuts and soybeans, but remarkable loss was also reported in maize, which was 31% and 19% in staple crop wheat (Gharde *et al*., 2018). Another study on dry beans showed a significant loss in yields due to the uncontrolled growth of weeds in that area (Soltani *et al*., 2018). The limitation in soil moisture content is directly related to transcription. And the loss of water due to transpiration is directly related to the canopy and biomass production (Song *et al*., 2020). In agricultural practices, there is a huge competition among weeds and crops for primary resources such as water, sun, light, and nutrients present in the soil. Other than that, the limitation in space is also a noticeable problem for crops due to uncontrolled weed production (Kaur *et al*., 2018). Other than yield loss due to weeds, disturbance in water-irrigated conditions for high costs is also a big problem because uncontrolled weed growth increases irrigation costs due to huge water losses (Singh *et al*., 2022). The presence of *parthenium* had a consistently negative impact on the growth and biomass of broccoli, lettuce, and peas across all sampling stages (Table 3). Chlorophyll a, b, total chlorophyll, carotenoids, as well as gas exchange parameters, showed a significant reduction in plants grown with *parthenium*. Our findings align with previous studies demonstrate that high weed density reduces the net photosynthetic rate through both stomatal and non-stomatal limitations. Weed competition was found to decrease the lamina area and stomatal density of wheat flag leaves while increasing the specific leaf area, ultimately impairing photosynthetic efficiency (Iqbal & Wright, 1999). Similarly, the presence of weeds has been reported to cause a continual decline in $CO_2$ assimilation and photosynthetic efficiency in common bean (*Phaseolus vulgaris* L.) (McKenzie-Gopsill *et al*., 2020). Moreover, intense competition for water between weeds and crops results in lower soil moisture content. This leads to stomatal closure and turgor loss, further limiting photosynthesis in crop plants (Freitas *et al*., 2019).

Osmolytes, including proline, soluble sugars, and free amino acids, play a crucial role in plant adaptation to both biotic and abiotic stresses. These compounds help maintain osmotic balance, stabilize proteins and membranes, and scavenge reactive oxygen species (ROS) under stress conditions (Jogawat, 2019). In the present study, parthenium-induced stress significantly influenced osmolyte accumulation in lettuce, broccoli, and peas, with the highest accumulation observed in lettuce across all measured time points. These findings align with previous studies reporting increased proline and sugar accumulation under competitive stress conditions. These results are consistent with past reports indicating that proline serves as a key stress marker, especially in plants exposed to allelopathic stress (Bakhshayeshan-Agdam & Salehi-Lisar, 2020). The increase in soluble sugars suggests an adaptive response to maintain osmotic balance, as noted in studies on abiotic stress tolerance in various crops (Afzal *et al*., 2021). The significant increase in proline, sugars, and amino acids under Parthenium stress highlights their role in stress mitigation.

Furthermore, our study revealed that *P. hysterophorus* exhibited significantly higher transpiration losses compared to broccoli, lettuce, and peas when grown independently. Notably, the evapotranspiration rate of vegetable crops increased when they were co-grown with *parthenium*, indicating intensified water competition under mixed growth conditions. This was especially evident in lettuce, where the water loss was significantly elevated at 25 DAS in the presence of *parthenium*. These findings, recorded at seven-day intervals, suggest that *parthenium* not only uses more water but also induces stress in neighboring crops by altering the water balance. Crop yield typically declines with increasing weed biomass due to competition for water, nutrients, and light (Kaur *et al*., 2018). Despite limited prior data on the water relations of *parthenium*, recent research shows that it can complete its life cycle at soil moisture levels as low as 50% of field capacity (Bajwa *et al*., 2017) a trend also observed in our study. This physiological flexibility enables *parthenium* to outcompete other species even in water-stressed conditions. Additionally, its ability to persist and reproduce in such environments makes it a growing agricultural threat. Our findings are consistent with those of (Adamson & Bray, 1999; Nguyen *et al*., 2017; Bajwa *et al*., 2020) confirming that *parthenium* significantly exacerbates water stress in cohabiting crops, reducing photosynthetic rates and overall productivity. Our study aligns with these findings, demonstrating its competitive nature, which exacerbates water stress, reduces crop photosynthetic efficiency, and negatively impacts productivity. Furthermore, the application of a fuzzy logic model allowed for a nuanced interpretation of the complex interactions between Parthenium presence, water dynamics, and physiological stress. This modeling approach provided more flexible analysis compared to traditional binary methods, offering a more accurate understanding of how Parthenium alters water budgeting in invaded ecosystems.

## Conclusion

*Parthenium hysterophorus* demonstrates a high competitive ability by reducing the biomass and growth of co-cultivated crops such as broccoli, lettuce, and peas. Our study confirms that *parthenium* significantly impairs physiological functions, particularly photosynthetic pigments (chlorophyll a, b, total chlorophyll, and carotenoids) and gas exchange traits, while increasing soil water loss and evapotranspiration (ETc). Moreover, the study introduces a methodological framework combining field data and fuzzy logic modeling to assess water budgeting under weed interference, offering a novel approach to quantify impact of *parthenium*. Additionally, osmolyte analysis revealed stress-induced changes in crop biochemistry under mixed growth conditions. These findings underline the urgent need for effective management strategies to mitigate ecological and agricultural threat of *parthenium*.

## Acknowledgement

This work is part of the Ph.D. thesis research conducted by Mr. Muhammad Zeeshan Arshed at the Department of Botany, University of the Punjab, Lahore.

**Authors Contribution:** M.Z.A. conceived and designed the study, performed the experiments, collected and analyzed the data, and drafted the initial version of the manuscript. M.S. supervised the research, provided guidance on experimental design, contributed to data interpretation, critically reviewed the manuscript, and approved the final version for submission.

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this paper.

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**1. Normality analysis of above-ground parameters for 250 Parthenium samples**

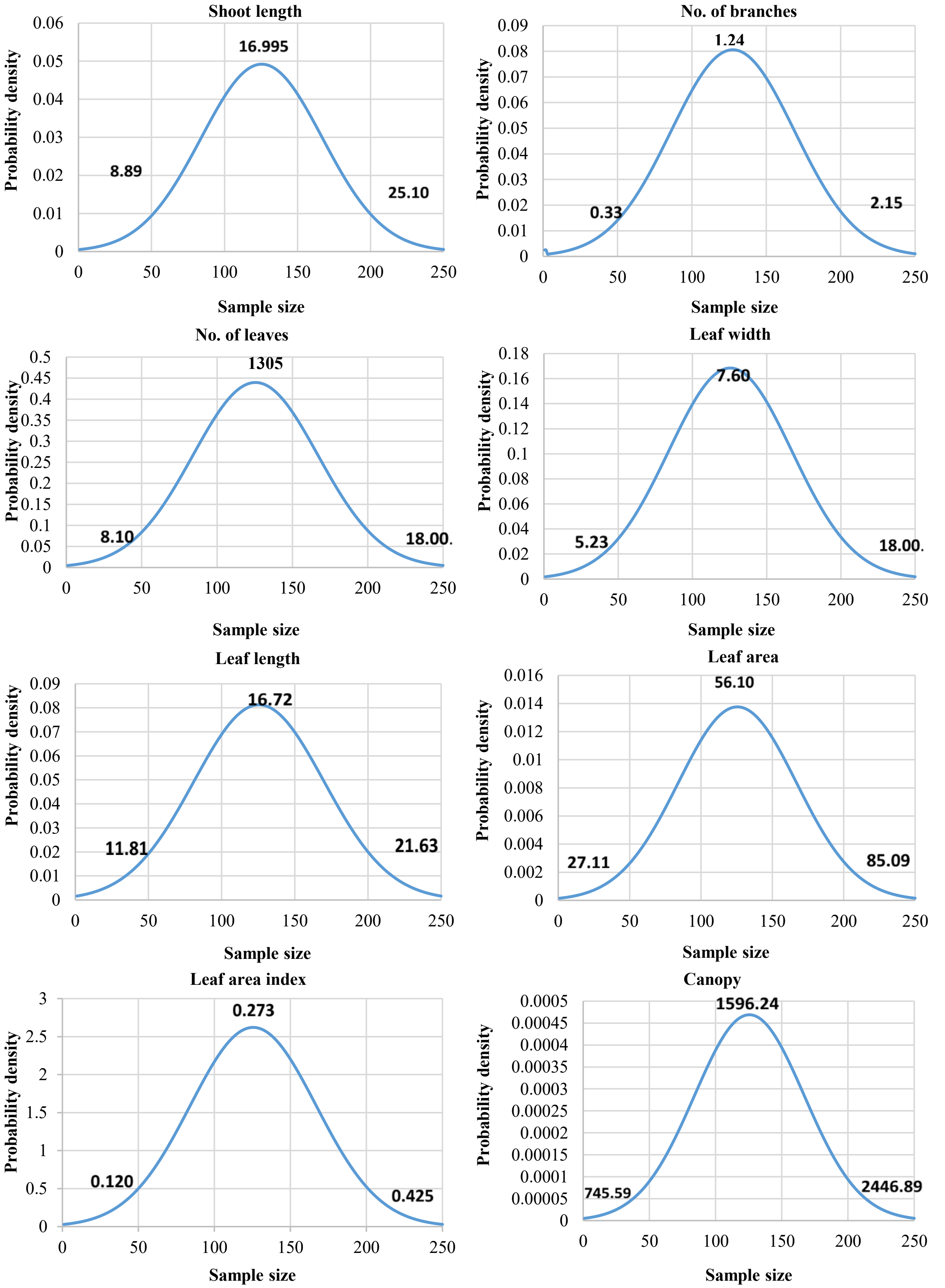



Fig. S1, The normality analysis of above-ground traits in 250 Parthenium samples showed that all parameters followed a normal distribution. The traits analyzed include shoot length, number of branches, number of leaves, leaf width, leaf length, leaf area, canopy cover, and leaf area index.

### 1.2. Normality analysis of below-ground parameters and plant height for 250 Parthenium samples

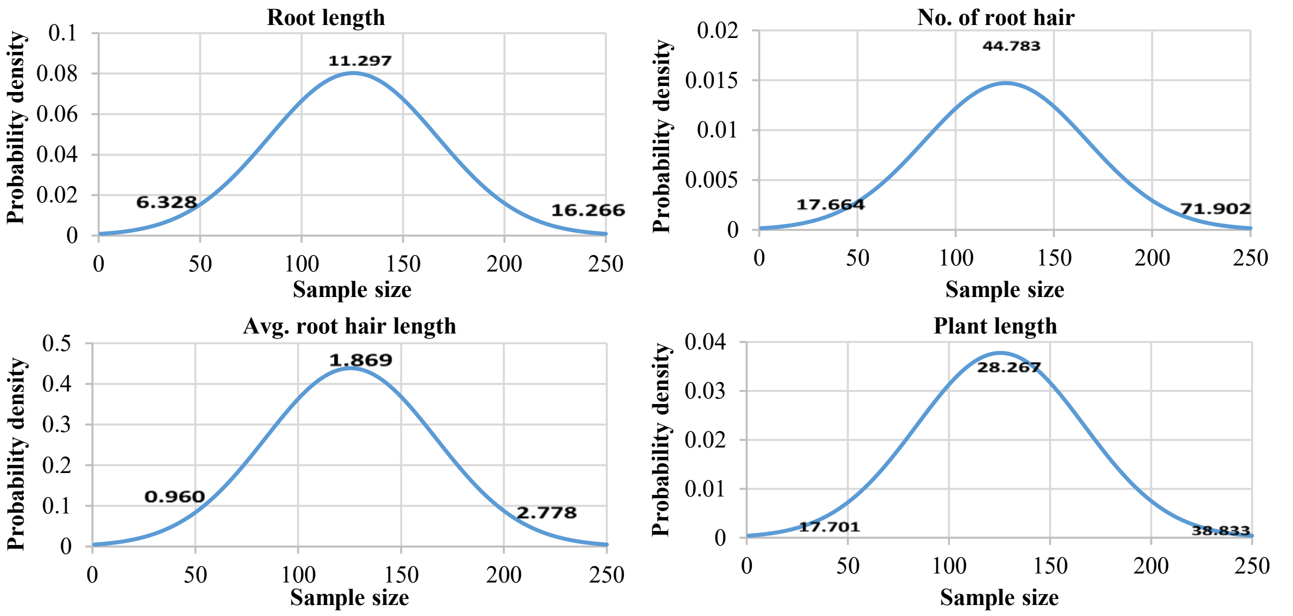



Fig. S2. Normality graphs illustrating the distribution of below-ground traits (root length, number of root hairs, and average root hair length) and total plant length in 250 Parthenium samples. All parameters followed a normal distribution, reflecting consistent below-ground and overall plant growth patterns across the population.

### 1.3. Normality analysis of ratio of shoot length to above-ground parameters in parthenium samples

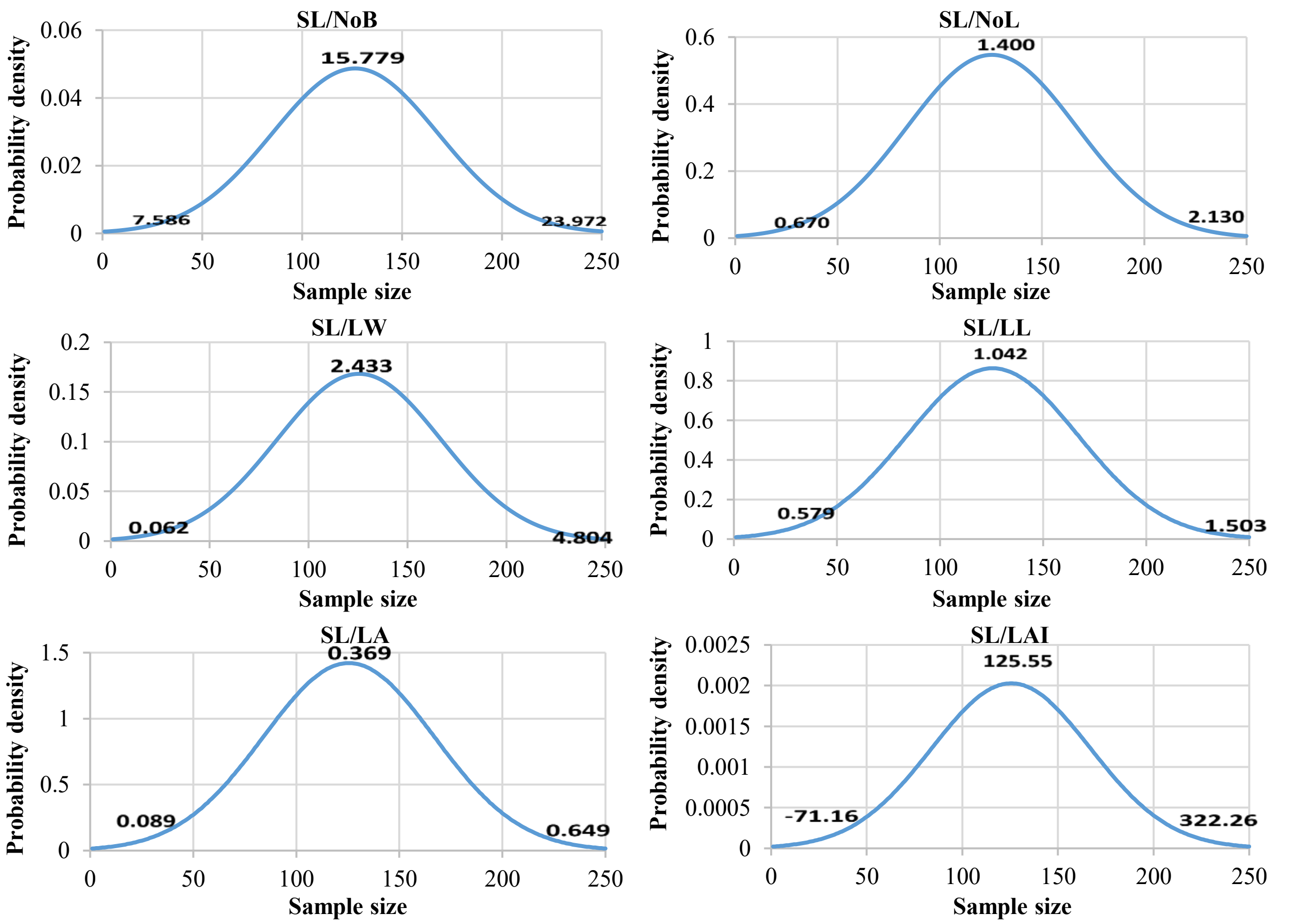



Fig. S3. Normality graphs illustrating the ratios of shoot length to above-ground parameters (number f branches, number of leaves, leaf width, leaf length, leaf area, and leaf area index) in 250 Parthenium samples. All ratios followed a normal distribution, highlighting consistent relationships between shoot growth and above-ground traits.

### 1.4. Normality analysis of ratio of shoot length to below-ground parameters and plant length in parthenium samples

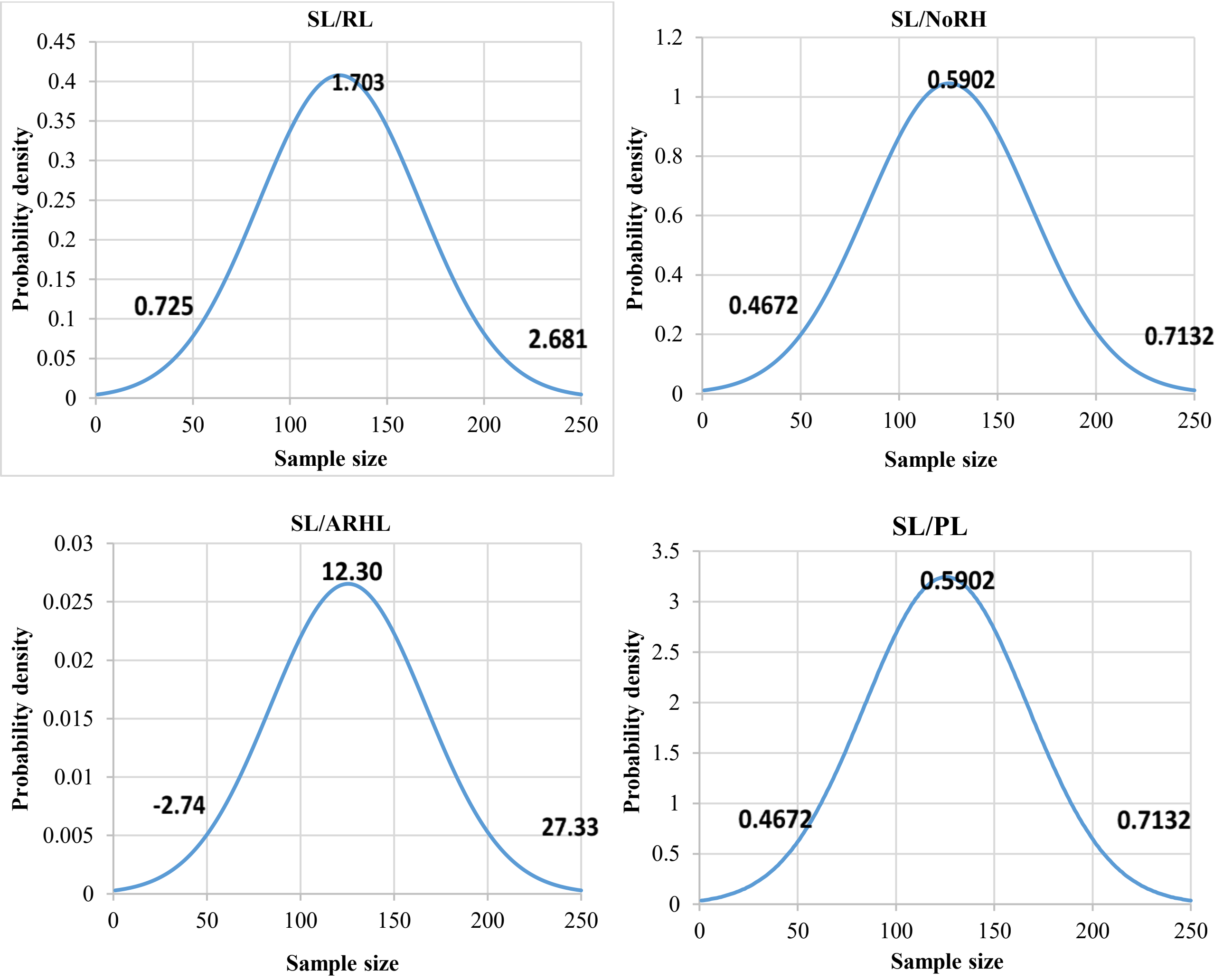



Fig. S4. Normality graphs illustrating the ratios of shoot length to below-ground parameters (root length, number of root hairs, average root hair length, and total plant length) in 250 Parthenium samples. All ratios followed a normal distribution, indicating consistent shoot-to-root allocation patterns across the population

### 1.5 Normality analysis of ratio of root length to below-ground parameters in parthenium samples

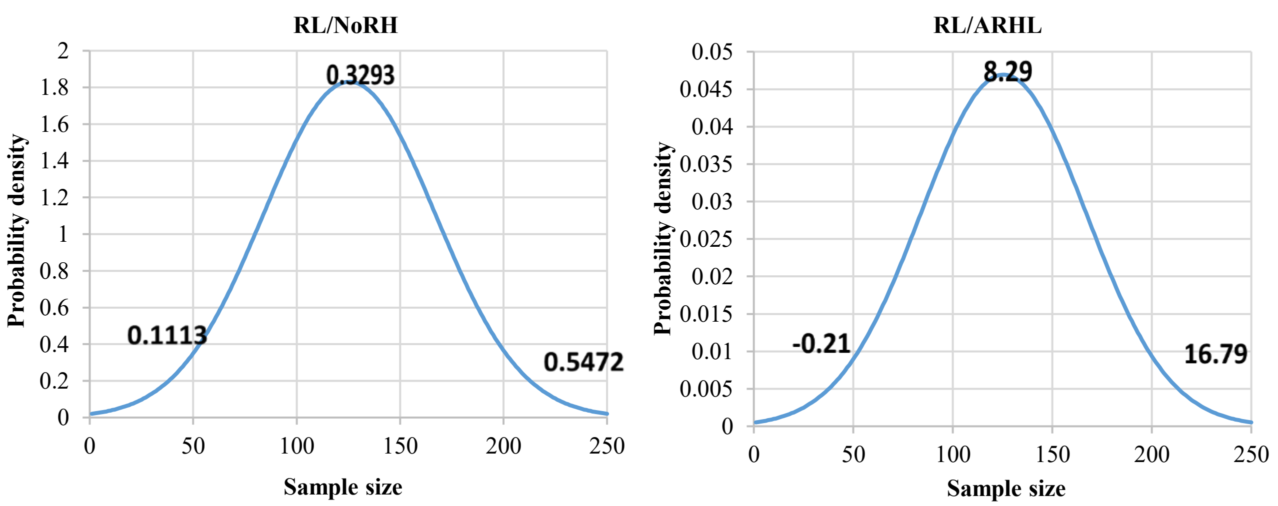



Fig. S5. Normality graphs illustrating the ratios of root length to below-ground parameters (number of root hairs and average root hair length) in 250 Parthenium samples. All ratios followed a normal distribution, reflecting consistent root growth and hair development patterns across the population.

**1.6. Normality analysis of fresh biomass parameters in parthenium samples**

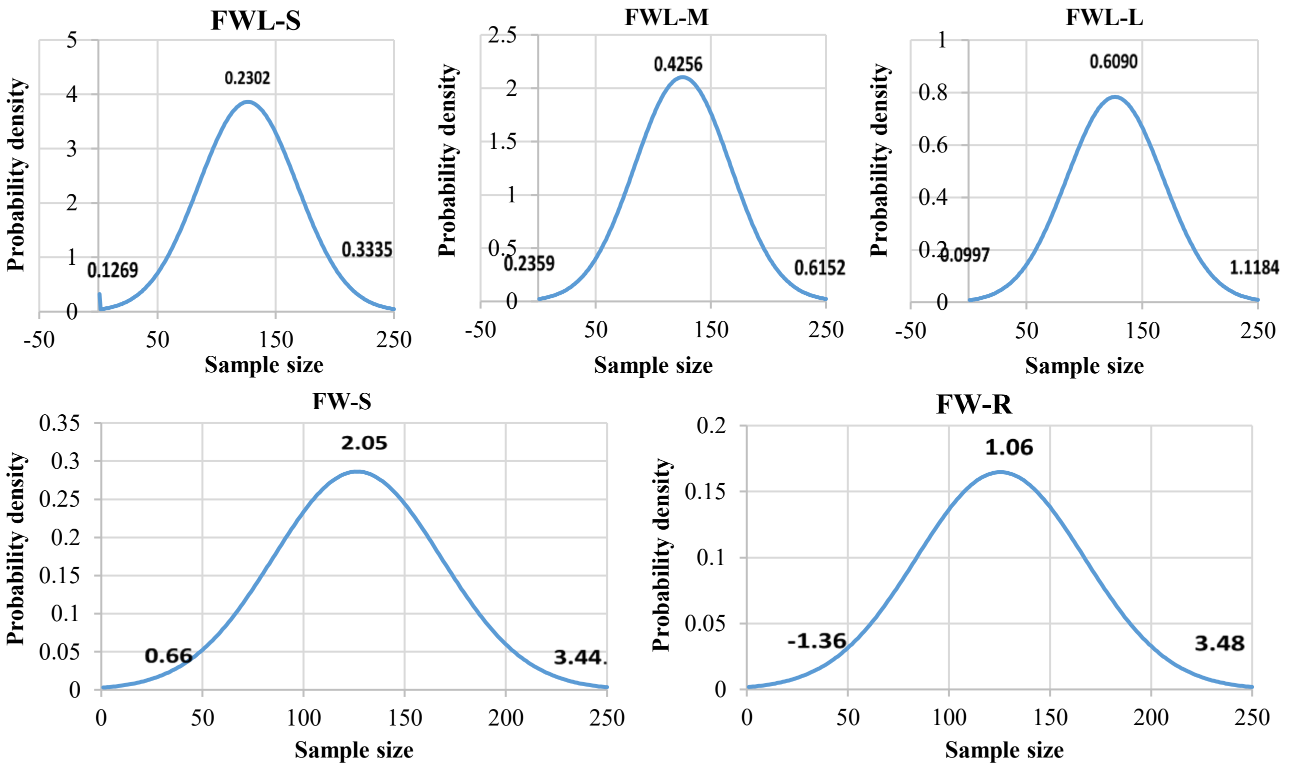



Fig. S6. Normality graphs illustrating the distribution of fresh weight parameters (small, medium, and large leaves, shoot, and root fresh weight) in 250 Parthenium samples. All parameters followed a normal distribution, demonstrating consistent biomass allocation across different plant components.

**1.7 Normality analysis of dry biomass parameters in parthenium samples**

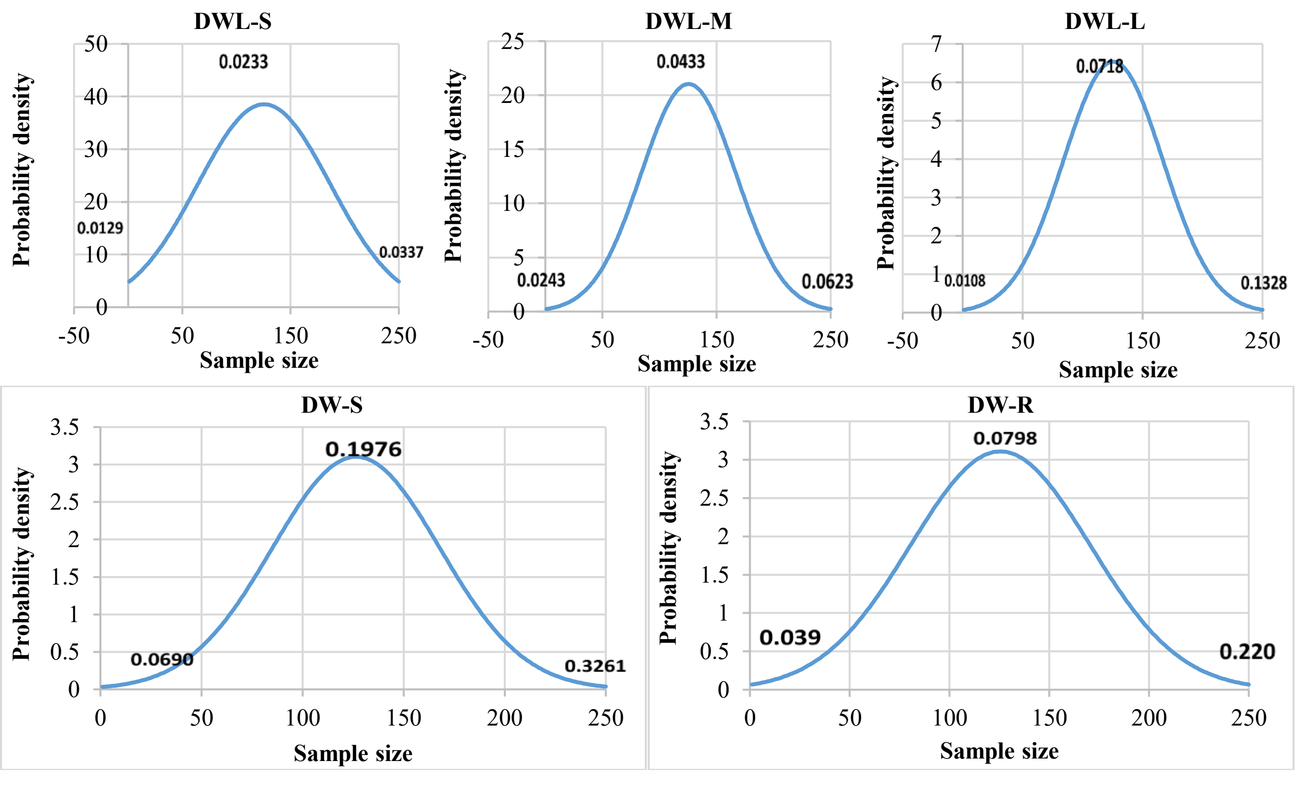



Fig. S7. Normality graphs illustrating the distribution of dry weight parameters (small, medium, and large leaves, shoot, and root dry weight) in 250 Parthenium samples. All parameters followed a normal distribution, demonstrating consistent biomass allocation trends across plant components.

### 1.8. Normality analysis of SPAD in parthenium samples

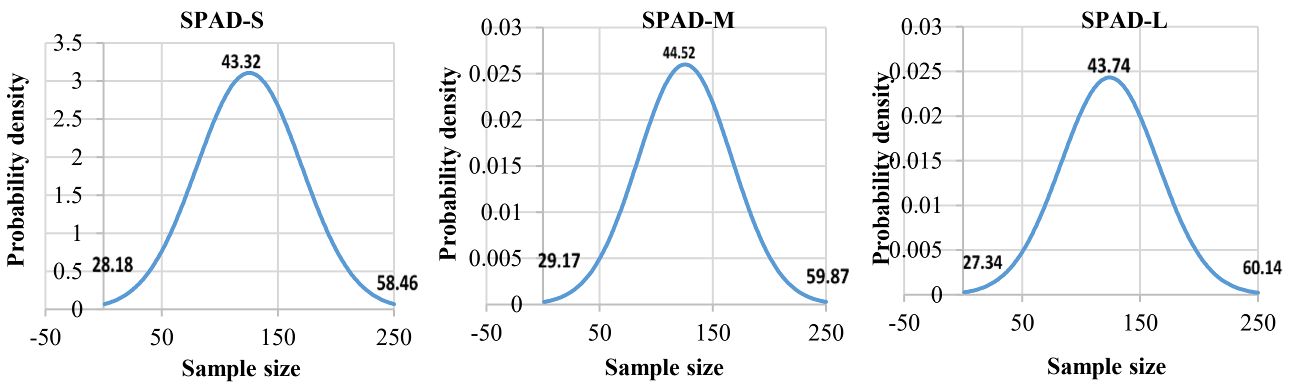



Fig. S8 Normality graphs illustrating the distribution of SPAD values in small, medium, and large leaves of 250 Parthenium samples. All SPAD values followed a normal distribution, indicating consistent chlorophyll content across different leaf sizes.

### 1.9 Relationship between RL/NoRH and above-ground parameter ratios in parthenium samples

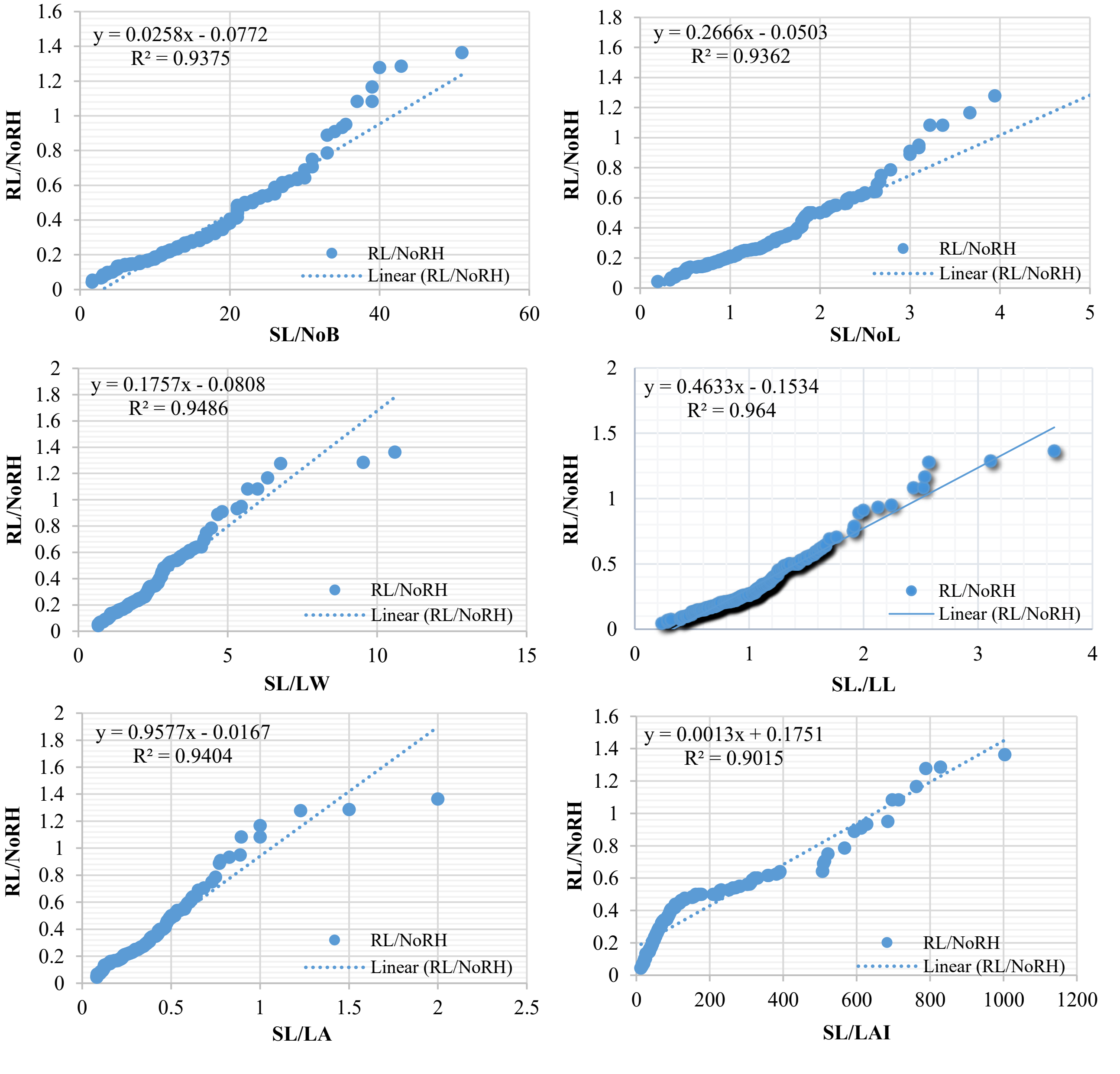



Fig. S9. Correlation between RL/NoRH and above-ground parameter ratios (SL/NoB, SL/NoL, SL/LW, SL/LL, SL/LA, SL/LAI) in parthenium samples.

**1.10. Relationship between RL/ARHL and above-ground parameter ratios in parthenium samples**

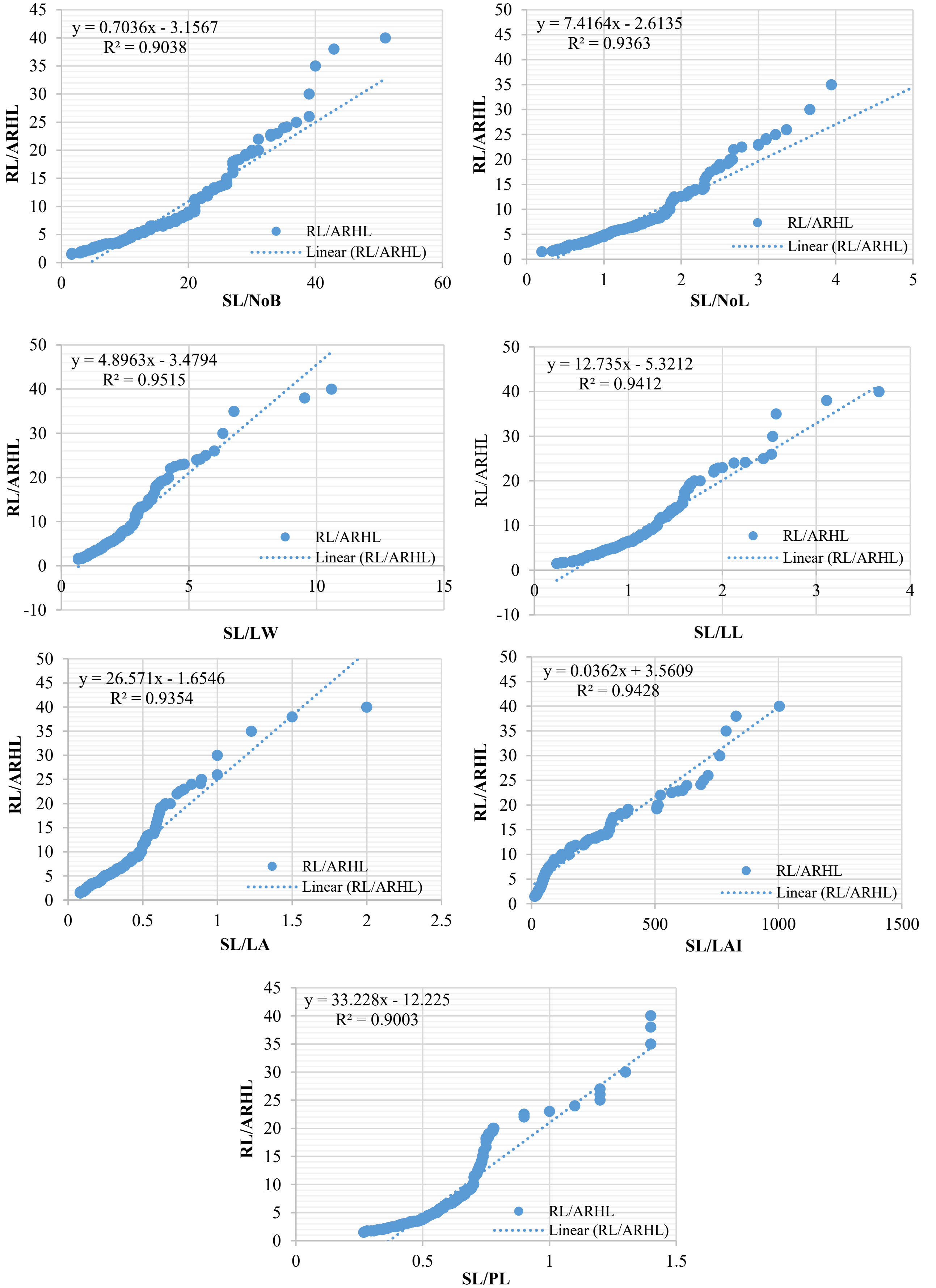
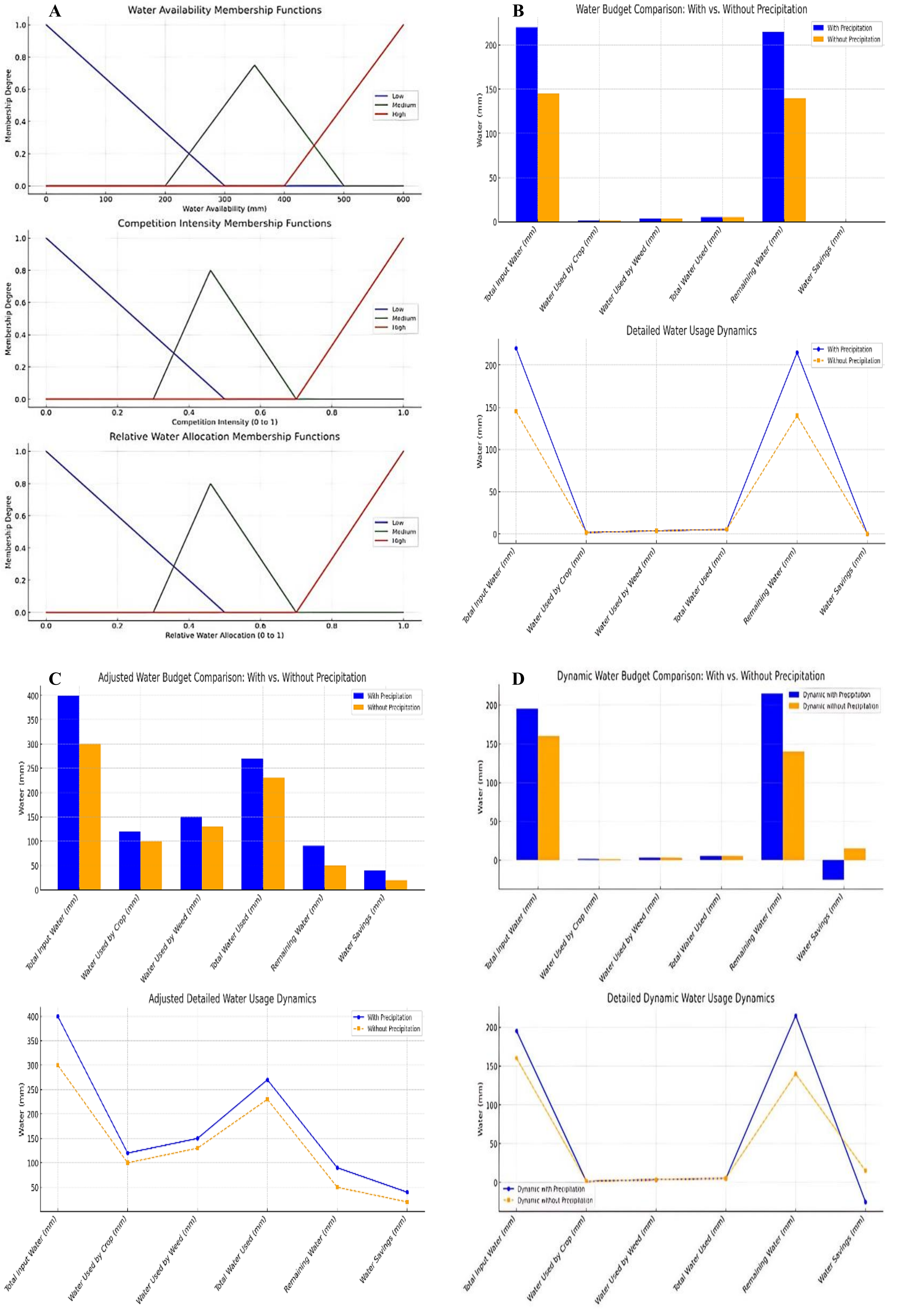



Fig. S10. Correlation Between below ground ratio RL/ARHL and above-ground parameter ratios (SL/NoB, SL/NoL, SL/LW, SL/LL, SL/LA, SL/LAI, SL/PL) in parthenium samples.


A
Water Availability Membership Functions
Membership Degree
Water Availability (mm)
Low
Medium
High
Competition Intensity Membership Functions
Competition Intensity (0 to 1)
Relative Water Allocation Membership Functions
Relative Water Allocation (0 to 1)
B
Water Budget Comparison: With vs. Without Precipitation
With Precipitation
Without Precipitation
Water (mm)
Total Input Water (mm)
Water Used by Crop (mm)
Water Used by Weed (mm)
Total Water Used (mm)
Remaining Water (mm)
Water Savings (mm)
Detailed Water Usage Dynamics
C
Adjusted Water Budget Comparison: With vs. Without Precipitation
Adjusted Detailed Water Usage Dynamics
D
Dynamic Water Budget Comparison: With vs. Without Precipitation
Dynamic with Precipitation
Dynamic without Precipitation
Detailed Dynamic Water Usage Dynamics

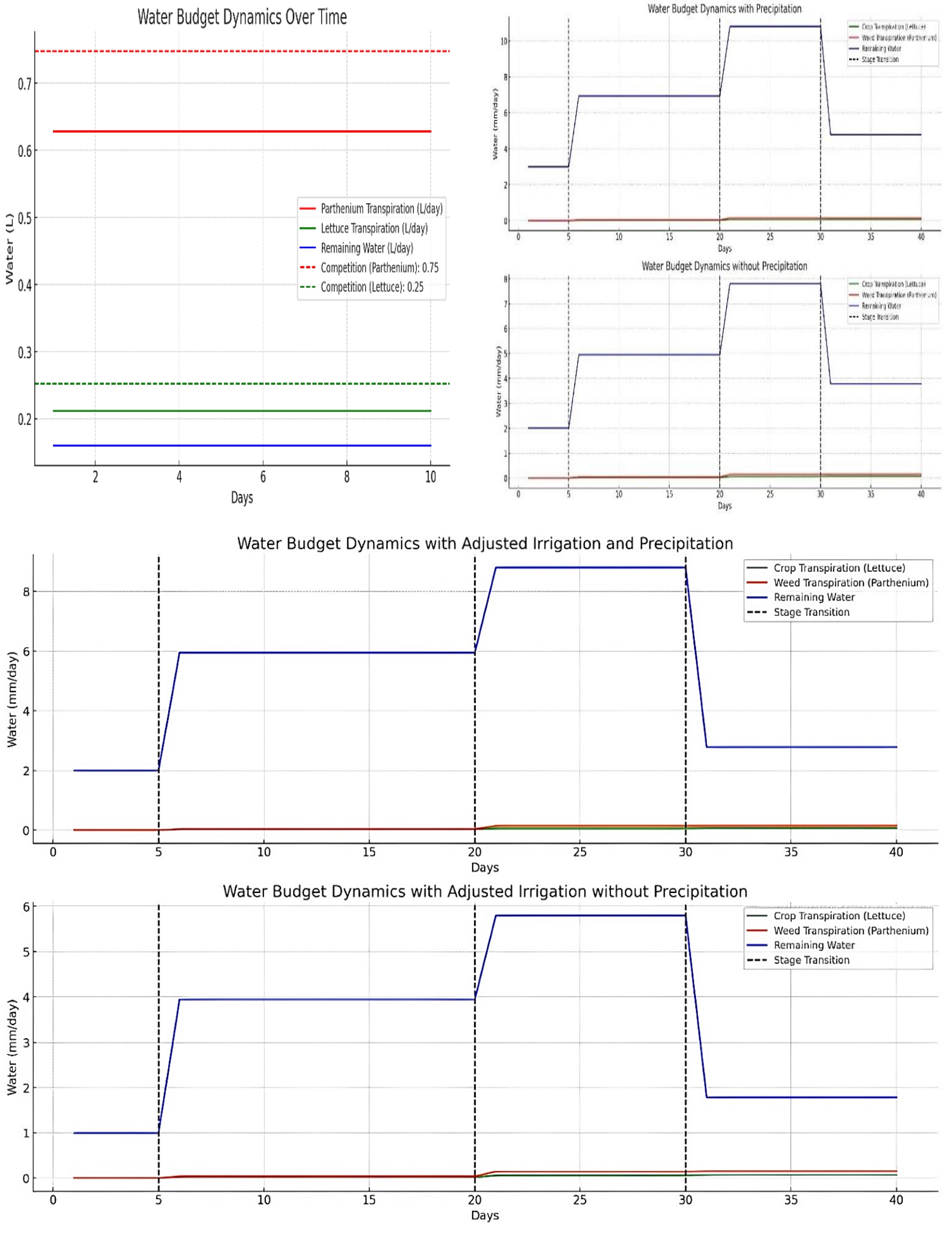



Fig. S11. Fuzzy logic model predictions for water availability and competition dynamics in a co-cultivated system of lettuce and parthenium (A) membership functions defining water availability, competition intensity, and relative water allocation (B) impact of precipitation on total water input, remaining water, and water savings (C) water availability trends with and without precipitation (D) comparison of static irrigation and precipitation effects on water use efficiency (E) water budget dynamics, showing daily water consumption by parthenium (red) and lettuce (green), with remaining water (blue) (F) influence of precipitation on water availability and transpiration rates, where parthenium exhibits higher water uptake (G) adjusted irrigation and precipitation effects on water balance, maintaining optimal water availability and reducing competitive water loss.