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Unveiling the Allelopathic Potential of Wedelia Leaf Extract as a Bioherbicide against Purple Nutsedge: A Promising Strategy for Sustainable Weed Management

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Abstract: Weed management is a crucial aspect of sustainable agriculture. In this study, we investigated the allelopathic potential of wedelia (*Wedelia trilobata* L.) leaf extract as a bioherbicide against purple nutsedge (*Cyperus rotundus* L.). The experiments were carried out through greenhouse experiments using a completely randomized design (CRD) with four replications. Five different concentrations were evaluated: C10% (10% wedelia extract concentration), C20% (20% wedelia extract concentration), C40% (40% wedelia extract concentration), C+ (92 mg L^{-1} of gallic acid), and C− (aquadest). Allelochemicals present in the wedelia leaf extract inhibited plant height, shoot number, leaf number, leaf area, root area, and total root length. The fresh weight, dry weight, and photosynthetic pigments decreased with increasing wedelia leaf extract concentrations. Malondialdehyde contents were highest when C40% was used. Additionally, peroxide activities decreased at the highest wedelia leaf extract concentration, indicating the failure of the plant's antioxidant defense mechanism. The decrease in growth, photosynthetic pigment, and antioxidant activity indicates that wedelia leaf extract may be able to help control the growth of purple nutsedge. The results of this study could contribute to the development of a new cropping system based on the use of wedelia as a bioherbicide for sustainable agriculture.

Keywords: allelopathy; *Cyperus rotudus* L.; plant-based herbicides; sustainable weed control; *Wedelia trilobata* L.

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1. Introduction

The world's population reached 8 billion in November 2022 [1], and it is projected to reach 9.8 billion in 2050 [2]. This massive population boom creates a significant challenge for agriculture and allied industries in meeting the world's increasing food demand and requires further increases in agricultural inputs [3]. In the future, the agro-tech industry is expected to face several challenges in ensuring food production for the world's fast-rising population. Having sufficient amounts of safe, nutritious food is a key factor in maintaining life and fostering well-being [4]. Between 2010 and 2050, global food consumption is expected to increase by 21% and it is estimated that the proportion of the population at risk for hunger will increase by 17%. Climate change also has significant implications for food demand, with an expected increase of 32%. The global community must adopt innovative and enhanced agricultural practices that prioritize sustainability and productivity to address these challenges [3].

Agricultural losses can be caused by abiotic and biotic factors, which decrease crop quality and actual crop yield compared to location-specific attainable crop yield and production [5]. Weeds are widely recognized as the most damaging biotic constraint to agricultural production [6] and cause the greatest potential loss (34%), followed by animal pests (18%) and pathogens (16%). In soybean production, weed competition threatens over

37% of global productivity, compared to pathogens (11%), viruses (1%), and animal pests (11%) [5].

Purple nutsedge (*Cyperus rotundus* L.) is one of the world's most harmful weeds. Its ability to grow rapidly and thrive in harsh conditions is attributed to the longevity and viability of its deep-seated tubers and rhizomes. The widespread presence of this plant in both arable and nonarable fields poses unique challenges for farmers and land managers since purple nutsedge can negatively impact crop yields and native plant communities [7]. The C4 metabolic pathway and reproductive strategy of vegetative propagation provide significant advantages to plant species in environments with elevated temperatures and light intensities. These adaptations allow plants to efficiently capture and utilize carbon dioxide, outcompete other plants, and reproduce rapidly to ensure their survival and dominance in various ecosystems worldwide [8]. The presence of purple nutsedge in agricultural fields negatively impacts crop growth and yield and harms agricultural productivity by directly competing with crops, interfering with crop production via allelopathy, and functioning as a substitute host for pests and diseases [9].

Purple nutsedge is economically damaging and has caused yield reductions of 20–90% in a variety of agronomic and horticultural crops around the world [9]. For example, purple nutsedge belowground interference decreased tomato shoot dry mass by 18% [10]. Additionally, it has caused significant yield losses of 23–89% in South Asian summer crops [11]. When weed control methods are delayed by 8 weeks, 60–80% of the total weed population found in sugar cane is purple nutsedge, particularly in the moisture-retentive soils of the tropics and subtropics, which significantly limits water and nutrient availability and leads to major crop yield losses (20–30%) [12]. In Mississippi, USA, it reduces cotton yields by up to 70–85% at high densities, resulting in a USD 40.5 million annual loss [13].

Possible control options for purple nutsedge management include cultural, physical, chemical, biological, and integrated management strategies. Since purple nutsedge is able to rapidly regenerate via new shoots, cultural practices are thought to be of limited value in their control. Although all of these techniques suppress purple nutsedge growth under certain conditions, they do not destroy the subterranean tuber network, which makes these methods unsuitable for long-term control. Further study is needed to investigate the long-term efficacy of mechanical and combination control approaches that integrate mechanical control with chemical alternatives in multiple cultivation systems. Furthermore, the impact of climate change on herbicidal efficacy is a key consideration when studying herbicide absorption, translocation, and metabolism in the control of purple nutsedge. Furthmore, non-target plant species can be harmed by the repeated application of nonselective herbicides over time, leading to the evolution of resistant biotypes. In addition, increased herbicide use results in higher production costs, which may be unaffordable for small landholders, and increased risks to human health and the environment. Most herbicides are ineffective against purple nutsedge because they do not kill dormant tubers. Thus, there is a need to develop an alternative, cost-effective strategy for controlling purple nutsedge while keeping environmental concerns in mind [9]. Chemical control using bioherbicide represents a viable strategy to ensure environmental safety and the cultivation of high-quality crops [14].

Researchers have verified the allelopathic potential of numerous plant and microbe residues and extracts in inhibiting the development and growth of purple nutsedge [7,15,16]. The substantial levels of chemical compounds that these extracts and residues add to the soil eventually inhibit the growth and development of this weed. The application of these allelochemicals might reduce the demand for toxicological chemical herbicides in purple nutsedge management, which would lower the risk of herbicide resistance and encourage the use of sustainable weed control strategies, especially in organic vegetable production systems [9].

The need for secure agricultural production and sustainable methods for weed control drives scientists to devise new approaches. New herbicides with safer environmental and

toxicological profiles have become increasingly necessary. Natural compounds offer a diverse range of potential new natural herbicides known as bioherbicides, which are based on compounds produced by living organisms [17]. Bioherbicides are products derived from living organisms or their secondary metabolites that can inhibit target weed populations without causing environmental damage [18]. Among the proposed approaches, research on allelopathy has recently become more prevalent in agricultural weed control [19]. Allelopathy, with its broad spectrum of advantages, may be a promising approach to mitigate environmental pollution and herbicide resistance evolution [20]. The natural phenomenon of allelopathy has been known for a long time and occurs when organisms impact the functioning of other nearby organisms in a negative or positive manner by releasing secondary metabolites [21]. Allelochemicals, or biologically active metabolites exuded by higher plants, fungi, and microorganisms, have phytotoxic properties that can be used to control weeds [4].

Wedelia (*Wedelia trilobata* L.) is a dangerous invasive weed that harms nearby vegetation via allelopathy. The plant belongs to Asteraceae (i.e., the sunflower family) and can spread quickly through vegetative propagation. The allelopathic chemicals of wedelia plants damage agricultural plants via a variety of bioactive compounds with different biological activities [22]. For example, diterpene, sesterpene, coumarin derivatives, and sesquiterpene lactones have been found in wedelia leaf extract. These compounds may contribute to the allelopathic effect of wedelias [23]. Additionally, wedelia leaf water extract was found to contain alkaloids, diterpenoid, and monosaccharides, which were found to have allelopathic effects [22].

Several studies have highlighted wedelia's allelopathic potential and its impact on other plants. For example, the activities of some hydrolytic enzymes and some protective enzymes in geminating rice seeds were reduced by aqueous wedelia root, stem, leaf, and whole plant extracts. Geminating with wedelia increased membrane permeability, decreased respiratory rate, and increased vitality. Physiological parameters were all reduced by aqueous wedelia extracts. The activities of nitrate reductase and glutamine synthetase, as well as the total nitrogen content in the leaves, were also significantly reduced by the extracts [24]. Wedelia water extract at concentrations ranging from 25% to 100% have been shown to decrease germination percentage, shoot length, and total chlorophyll content in rape plants (*Brassica campestris* L.). These findings suggest that wedelia has strong phytotoxic effects on rape plants [25]. Wedelia water and crude extract also have been shown to affect lettuce seed germination and root growth [26]. Wedelia extract significantly inhibits the growth of pulse seedlings, with a greater impact at higher concentrations [23]. This study explores the allelopathic potential of wedelia leaf extract as a bioherbicide against purple nutsedge for sustainable weed management.

2. Materials and Methods

2.1. Experimental Site

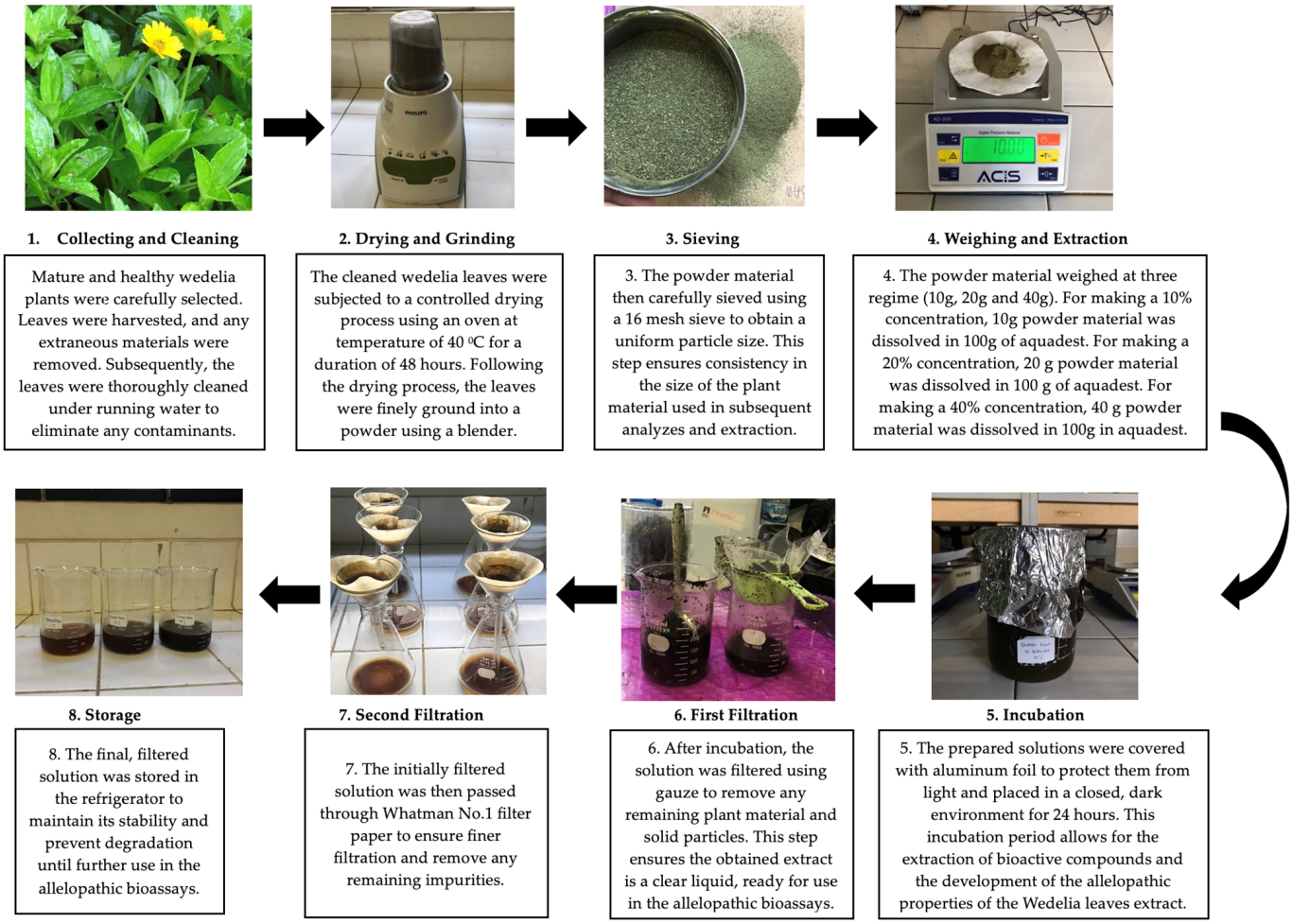
The field experiment was conducted from April to August 2023 at the experimental field of the Agricultural Faculty of Universitas Gadjah Mada, Banguntapan, Bantul District, Special Region of Yogyakarta Province, Indonesia (07°48′17″ S, 110°24′45″ E). Purple nutsedge tubers and wedelia leaves were obtained from the Bantul District of Indonesia from April to June 2023. The experiments were carried out through greenhouse experiments.

2.2. Experimental Design

The experiment was conducted using a single-factor completely randomized design (CRD) with five different concentrations and four replications. The factor tested was the wedelia leaf aquadest extract concentration for application. The treatments consisted of five different concentrations (C−), which consisted of three wedelia leaf extract concentrations (C10%, C20%, and C40%), a reference compound of gallic acid (C+; 92 mg L^{-1} of gallic acid), and a control of aquadest (C−).

2.3. Extract Preparations

The process of extracting valuable compounds from wedelia leaves is meticulous and involves several sequential stages, as shown in Scheme 1.



Scheme 1. Steps to creat wedelia extract.

2.4. Tubers Planting

Purple nutsedge tubers were collected at Bantul District, Special Region of Yogyakarta Province, Indonesia (07°48′17″ S, 110°24′45″ E). The purple nutsedge tubers were washed and weighed to a uniform size and weight (±1 g). Forty tubers were planted per tray (35 cm × 26 cm × 5 cm) and each tray was filled with 500 g of sterile soil, which was dried in an oven at 45 °C before filling the trays to control and eliminate potential contaminants, pathogens, or unwanted organisms that could affect the study results. The experiment was conducted in four replicates. Each single treatment plot had three experimental units: a growth unit, a destruction unit, and a reserve unit.

2.5. Extract Application

Wedelia leaf extract was applied one day after the purple nutsedge tubers were planted in a ratio of 1:10 soil to solution (i.e., 500 g soil needed 50 mL solution) for each treatment concentration (C10%, C20%, C40%, C+, and C−) (Figure 1). The purple nutsedge was kept moist by adding 20 mL of each solution every two days for 40 days.

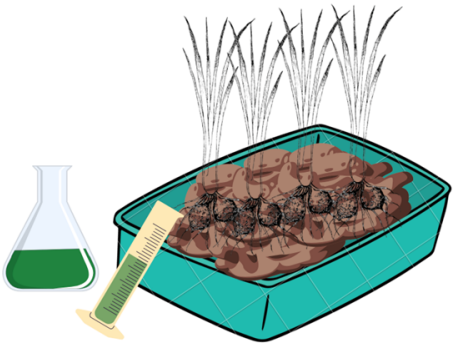


Figure 1. Illustration of the extract application through the root system.

2.6. Observations

2.6.1. Microclimate Condition

Microclimate condition observations were conducted at regular intervals of 10 days throughout the research period. The observations were made during different times of the day, specifically in the morning (6–7 a.m.), afternoon (12–13 p.m.), and late afternoon (16–17 p.m.) WIB (Western Indonesian Time). However, the data shown are only from the afternoon because it is considered the time of maximum photosynthesis. Temperature (°C) and air humidity (%) observations were performed using a thermohygrometer. Observations were carried out at a consistent height of 1 m above the plants.

2.6.2. Growth Parameters

The measured growth parameters included plant height (cm), number of shoots, number of leaves, leaf area (cm^2), root area (cm^2), total root length (cm), fresh weight (g), and dry weight (g). The growth parameters were estimated by measuring five randomly selected plants at 20 and 40 days after planting (DAP). Plant height was measured at 10, 20, 30, and 40 DAP. Leaf area, root area, and total root length analyses were performed using an area meter at 20 and 40 DAP. Leaf area analysis was conducted from a selection of representative plants. Briefly, leaves were harvested from the selected plants and cleaned to remove dirt and debris. The cleaned leaves were then arranged on the leaf area meter and the total leaf area was measured. Plants selected for root area and root length analysis were excavated from the soil by carefully removing dirt from the roots with a shovel while minimizing root damage. Once removed, the root systems were cleaned to remove excess soil and debris. The cleaned roots were allowed to dry, then arranged on the root area or length meters as applicable. The area meter was used to measure the root area or length. At the end of the experiment (day 40) the remaining plants were harvested and the fresh weight (g/plant) of each sample was measured with the help of an analytical weighing balance and recorded. The plants were then placed in an oven at 80 °C until a constant dry weight was obtained, which was also recorded as g/plant.

2.6.3. Physiological Parameters

The extraction, estimation, and determination of chlorophyll a, chlorophyll b, and total chlorophyll were carried out using the methods of Arnon (1949) [27] and Kaur (2016) [28], while carotenoids were determined using the Lichtenthaler (1987) [29] method. Briefly, fully-expanded leaves (0.5 g) were extracted using 10 mL of 80% (v/v) acetone. The leaves were ground in a mortar and filtered using Whatman filter paper. The absorbance was measured using a UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) at wavelengths of 663 mm, 645 mm, and 470 mm for chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids.

$$\text{Chlorophyll a} = \frac{((12.7 \times 663\ \mu\text{m}) - (2.69 \times 645\ \mu\text{m}) \times V)}{1000 \times W\ (0.5)}$$

$$\text{Chlorophyll b} = \frac{((22.9 \times 663\ \mu\text{m}) - (4.68 \times 645\ \mu\text{m}) \times V)}{1000 \times W}$$

$$\text{Total chlorophyll} = \frac{((20.2 \times 645\ \mu\text{m}) + (8.02 \times 663\ \mu\text{m}) \times V)}{1000 \times W}$$

$$\text{Total carotenoids} = ((1000 \times 470\ \mu\text{m}) - (1.82 \times chl\ a) - (85.02 \times chl\ b)) \times (\frac{V}{1000}) \times (\frac{1}{W}) \times (\frac{1}{198})$$

where

V: chlorophyll extract final volume in 80% acetone (10 mL);
W: fresh weight of the leaves (0.5 g).

2.6.4. Biochemical Parameters

The biochemical parameters measured in this experiment were malondialdehyde (MDA), hydrogen peroxide, and peroxide. The MDA contents were used to determine the lipid peroxidation in leaves and was performed according to the Senthilkumar (2021) method with modifications [30] using tiobarbituric acid (TBA). Briefly, fresh leaves (0.2 g) were weighed and ground using a mortar and pestle. The leaves were homogenized with 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution in a 15 mL centrifuge, then the mixture was centrifuged at 12,000 rpm for 15 min. The supernatant (1 mL) was added to a solution of 2 mL 0.5% TBA in 20% TCA. The mixture was shaken with a vortex and incubated in a water bath at 90 °C for 20 min. The test tube was cooled using cold water for 10 min to stop the reaction. The absorbance was measured using a spectrophotometer at wavelengths of 532 and 600 nm. To calculate the MDA levels in leaves, we used the molar extinction coefficient of 1.57×10^5, which describes the strength of light absorbance by MDA at wavelengths of 532 and 600 nm [31]. MDA levels are expressed in µmol per fresh weight (µmol MDA g^{-1} FW) and were calculated using the following equation:

$$\text{MDA content } (\mu\text{mol MDA g}^{-1}\text{ FW}) = \frac{(\frac{A_{532} - A_{600}}{157000} \times 10^6)}{W} \times V_t$$

where

A_{532}: extract absorbance at 532 nm; W: sample use in measurement (0.2 g);
A_{600}: extract absorbance at 600 nm; V_t: total volume reaction (3 mL);
1.57×10^5: molar attenuation coefficient MDA.

The activity of hydrogen peroxide (H_2O_2) was estimated according to the Zhou (2006) method [32] with modifications. Briefly, fresh leaves (0.5 g) were ground using a mortar with 3 ml of 0.1% (w/v) TCA. The mixture was centrifuged at 8000 rpm for 1 min at 4 °C. Then, 0.5 mL of a 100 mM potassium phosphate buffer (pH 7.4; 100 mM ≈ 2.12% w/v) and 2 mL of potassium iodide (KI) reagent (1 M ≈ 16.6% w/v in H_2O) were added to the reaction tube and 1 mL of supernatant was added. The mixture was allowed to sit for an hour in the dark, then the sample was placed in a cuvette and the absorption was read using a UV-visible spectrophotometer at a wavelength of 390 nm. The blank solution used was 1 mL of 5% TCA, 2 mL 1 M KI, and 100 mM of 0.5 mL potassium phosphate buffer. The H_2O_2 content is expressed in µmol H_2O_2 per fresh weight (µmol H_2O_2 g^{-1} FW) using the following equation:

$$H_2O_2 \text{ content } (\mu\text{mol } H_2O_2\ g^{-1}\text{ FW}) = \frac{H_2O_2 \text{ in sample reaction} \times V_t}{\text{Sample weight (g)}}$$

where

H_2O_2 in the sample reaction: H_2O_2 content value obtained from the standard curve;
V_t: total volume reaction (3.5 mL);
Sample weight: sample used in measurement (0.5 g).

The peroxide activity was estimated using pyrogallol as a substrate. Briefly, fresh leaves (1 g) were ground using liquid nitrogen until smooth, then 3 mL of a 100 mM (pH 7) phosphate buffer solution was added for homogenization. The extract was filtered with filter paper and placed into a 15 mL centrifuge tube, then centrifuged at 4000 rpm for 20 min at 4 °C. The reagents included aquadest, 100 mM phosphate buffer (pH 7) at a temperature of 20 °C, 12.3 mM H_2O_2 solution, 5% pyrogallol solution, and the supernatant. The 12.3 mM H_2O_2 solution was made from 30% H_2O_2 (0.14 mL of 30% H_2O_2 in 100 mL of aquadest) and stored at a low temperature. The reagent was placed into the cuvette first, and the supernatant was added quickly. The blank solution used included all reagents except the supernatant. The extract mixture was divided into blank cuvettes, control cuvettes, and sample cuvettes. Aquadest (2.1 mL), 0.32 mL of 100 mM phosphate buffer (pH 7), 0.16 mL of 0.5% H_2O_2 solution, and 0.32 mL of 5% pyrogallol were added to each cuvette. The mixture was homogenized by gentle shaking, then placed in sample holder number two. Next, 0.1 mL of the supernatant was placed into a cuvette and homogenized by gentle shaking.

The mixture had a yellow color due to the formation of purpurogallin in the mixture of buffer compounds with pyrogallol reagent and peroxidase [33]. Changes in absorbance were measured quickly using a spectrophotometer at 420 nm for 3 min every 30 s. The peroxide (POD) enzyme content is expressed in units per minute per fresh weight (unit min^{-1} g^{-1} BS). The POD enzyme content was calculated using the following equation:

$$\text{POD content (Unit min}^{-1}\text{ g}^{-1}\text{ FW)} = \frac{\left(\Delta A_{420\ nm,\ sample} - \Delta A_{420\ nm,\ blank}\right) \times Vt}{W \times 12 \times Vs \times t}$$

where

$\Delta A_{420\ nm,\ blank}$: increase in absorbance at wavelength 420 nm (blank);
$\Delta A_{420\ nm,\ sample}$: increase in absorbance at wavelength 420 nm (sample);
V_t: total volume reaction (3 mL);
V_S: sample volume (0.1 mL);
W: sample weight (1 g);
t: kinetic absorbance observation time (3 min);
12: molar attenuation coefficient of purpurogallin.

2.6.5. Tuber Anatomy

The anatomical details of a 40-day-old purple nutsedge plant were examined in this study according to Maiti (2012) method [7]. Briefly, the plants were uprooted from their pots and rinsed with tap water to remove any soil residue before analysis. Subsequently, serial transverse sections of were obtained of the tubers. A microscope was used to examine the anatomy of these sections from both treated and control plants. To document the anatomical features, the slides were captured with photographs.

2.7. Statistical Analysis

The experiment was arranged in a completely randomized design (CRD) with four replications. The data were tested for normality with the Shapiro–Wilk test and then analyzed using the analysis of variance (ANOVA). The data continued with honestly significant differences (HSDs) at the 0.05 probability level using RStudio software 4.3.2 version.

3. Results

3.1. Microclimate Condition

During the research period, the microclimate conditions were regularly monitored to provide insights into the environmental factors influencing the experimental setup. Temperature observations revealed consistent values throughout the study. The average temperature recorded during the observation period was 31.32 °C as shown in Table 1. Air

humidity, an essential parameter influencing plant growth, was closely monitored. The average air humidity recorded during the research period was 61.5% as shown in Table 1. These microclimate conditions were integral to understanding the ambient environment in which the experimental plants were exposed, contributing valuable insights to the broader ecological context of the study.

Table 1. Microclimate condition at 10, 20, 30, and 40 DAP.

DAP	Microclimate Condition	
	Temperature (°C)	Air Humidity (%)
10	30.4	69%
20	32.6	59%
30	30.1	63%
40	32.2	55%
Average	31.32	61.5%

3.2. Growth Parameters

3.2.1. Plant Height

The results show the effects of different wedelia leaf extract concentrations (10%, 20%, 40%) on plant height, number of shoots, number of leaves, leaf area, root area, and root length (Figure 2 and Table 2). The fresh wedelia leaf extract significantly decreased plant height compared to the control (Figure 2) The 40% concentration was the most inhibitory to plant height (Figures 2 and 3). The growth parameters' reduction trend was correlated to the applied extract concentra-tions, indicating that greater inhibitory effects were found at higher concentrations and less at lower concentrations. The plant height reached inhibition levels of 75% at 10 DAP, 61% at 20 DAP, 48% at 30 DAP, and 46% at 40 DAP for the highest concentration (40%).

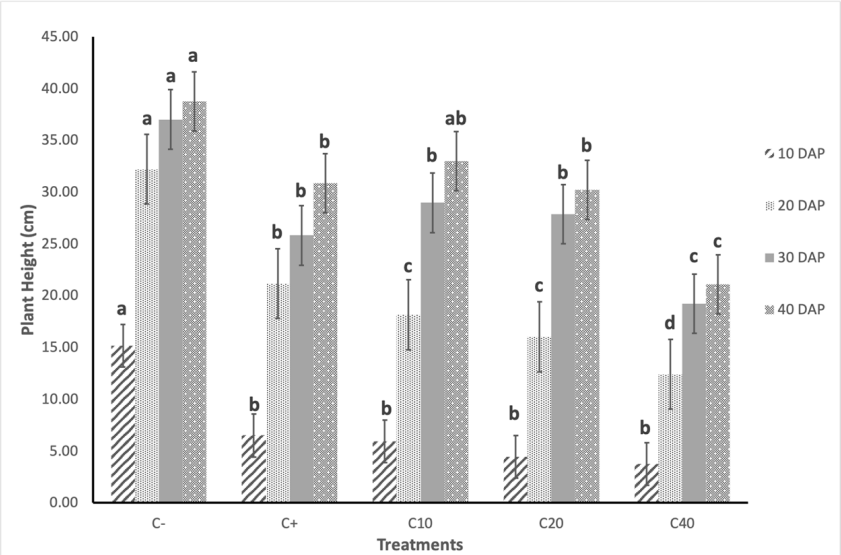
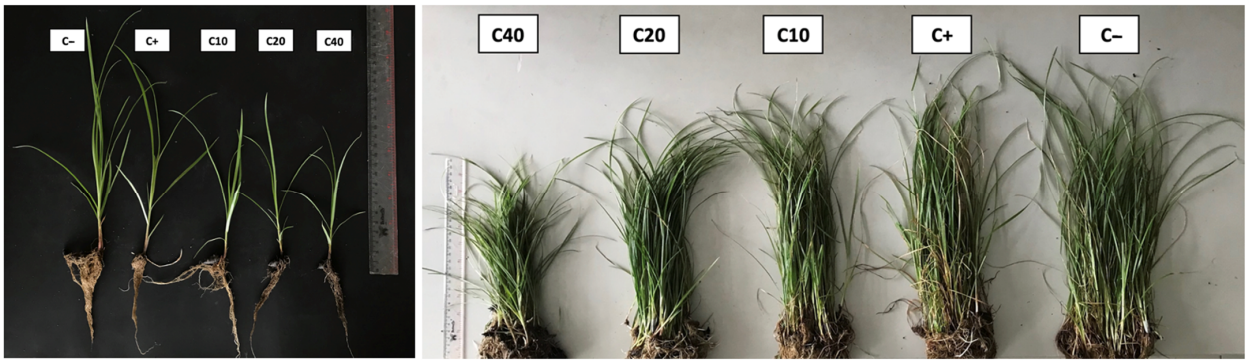


Figure 2. Effect of wedelia extract (*Wedelia trilobata* L.) on plant height of purple nutsedge (*Cyperus rotundus* L.). Mean values followed by the same letters are not significantly different at $p = 0.05$ (ANOVA and Tukey's test) and $n = 4$. The error bars indicate the standard error. C− = aquadest; C+ = gallic acid; C10 = 10% wedelia extract concentration; C20 = 20% wedelia extract concentration; and C40 = 40% wedelia extract concentration.

Table 2. Effect of wedelia extract (*Wedelia trilobata* L.) on the growth parameters of purple nutsedge (*Cyperus rotundus* L.) at 20 and 40 DAP.

Parameters	DAP	Treatments				
		C−	C+	C10	C20	C40
Number of shoots	20	83.25 ± 4.717 a	73.50 ± 5.000 a	73.00 ± 3.266 a	60.50 ± 7.594 b	60.25 ± 5.058 b
	40	90.00 ± 4.243 a	77.75 ± 4.646 ab	84.50 ± 5.802 ab	81.75 ± 11.615 ab	69.75 ± 7.544 b
Number of leaves	20	7.85 ± 0.191 a	5.60 ± 0.432 b	5.80 ± 0.163 b	5.40 ± 0.712 b	5.35 ± 0.619 b
	40	9.00 ± 0.283 a	8.05 ± 0.915 ab	8.55 ± 0.252 ab	7.80 ± 0.432 b	7.55 ± 0.551 b
Leaf area (cm^2)	20	31.28 ± 5.096 a	25.65 ± 1.955 a	17.11 ± 4.871 b	14.81 ± 2.975 b	10.03 ± 1.450 b
	40	89.53 ± 22.346 a	46.06 ± 13.295 b	41.05 ± 1.101 b	39.03 ± 16.636 b	27.93 ± 3.936 b
Root area (cm^2)	20	17.76 ± 3.716 a	15.33 ± 3.190 ab	12.37 ± 2.919 ab	11.70 ± 2.934 ab	9.28 ± 1.654 b
	40	29.51 ± 6.043 a	16.80 ± 4.436 b	14.93 ± 5.753 b	12.72 ± 3.935 b	9.93 ± 3.436 b
Total root length (cm)	20	32.20 ± 16.409 a	21.15 ± 11.505 b	18.15 ± 9.360 c	16.00 ± 5.883 c	12.40 ± 1.242 d
	40	38.75 ± 7.810 a	30.85 ± 14.385 b	32.95 ± 10.373 ab	30.20 ± 2.031 b	21.08 ± 7.736 c

Mean values followed by the same letters are not significantly different at $p = 0.05$ (ANOVA and Tukey's test) and $n = 4$. The error bars indicate the standard error. C− = aquadest; C+ = gallic acid; C10 = 10% wedelia extract concentration; C20 = 20% wedelia extract concentration; and C40 = 40% wedelia extract concentration.



(a) (b)

Figure 3. (**a**) Growth of purple nutsedge (*Cyperus rotundus* L.) at 20 DAP, and (**b**) growth of 20 tubers of purple nutsedge at 40 DAP. C− = aquadest; C+ = gallic acid; C10 = 10% wedelia (*Wedelia trilobata* L.) extract concentration; C20 = 20% wedelia extract concentration; and C40 = 40% wedelia extract concentration.

3.2.2. Number of Shoots and Leaves

The allelopathic leaves extract of wedelia showed a significant inhibition of the emergence of shoots. New shoots were inhibited at a higher concentration (40%) both at 20 and 40 DAP (Table 2). The wedelia extract decreased the number of leaves and hindered leaf promotion. The highest concentration (40%) caused the highest growth inhibition compared to the other concentrations (10% and 20%).

3.2.3. Leaf and Root Areas

Significant inhibition of the leaf number resulted in a decrease in leaf and root area. The highest inhibition was found in the wedelia extract concentration of 40% in both leaf and root areas at 20 and 40 DAP (Table 2).

3.2.4. Total Root Length

The wedelia leaf extract significantly affected root length in the purple nutsedge (Table 2). The lowest root length was recorded for the 40% concentration of wedelia extract. The highest values were in the control.

3.2.5. Fresh and Dry Weights

After 40 days of treatment, the wedelia leaf extract decreased the fresh and dry weight of the purple nutsedge. The highest inhibition was observed in the 40% wedelia extract concentration, while the lowest inhibition was observed in the 10% wedelia extract concentration. The highest wedelia extract concentration (40%) decreased the fresh and dry weight by 57% and 58%, respectively, compared to the controls (Table 3).

Table 3. Effect of wedelia extract (*Wedelia trilobata* L.) on the number of vascular bundles (VB), fresh weight, and dry weight of purple nutsedge (*Cyperus rotundus* L.) at 40 DAP.

Parameters	Treatments				
	C−	C+	C10	C20	C40
No. of VBs	33.00 ± 1.73 a	13.00 ± 1.73 c	17.00 ± 2.00 c	27.00 ± 2.00 b	24.00 ± 2.00 b
Fresh Weight (g)	1.33 ± 0.456 a	0.62 ± 0.172 b	0.59 ± 0.208 b	0.59 ± 0.052 b	0.57 ± 0.152 b
Dry Weight (g)	0.36 ± 0.116 a	0.19 ± 0.058 ab	0.18 ± 0.060 b	0.18 ± 0.022 b	0.15 ± 0.005 b

Mean values followed by the same letters are not significantly different at $p = 0.05$ (ANOVA and Tukey's test) and $n = 4$. The error bars indicate the standard error. C− = aquadest; C+ = gallic acid; C10 = 10% wedelia extract concentration; C20 = 20% wedelia extract concentration; and C40 = 40% wedelia extract concentration.

3.3. Physiological Parameters

The wedelia leaf extract had varied effects on the pigment content of the purple nutsedge at 40 DAP (Table 4). The experiment shows a decrease in chl a, chl b, total chl, and carotenoid content in the treated plants. The highest concentration of wedelia extract (40%) decreased the chl a, chl b, total chl, and carotenoid content at 31%, 29%, 43%, and 41%, respectively, compared to the controls.

Table 4. Effect of wedelia extract (*Wedelia trilobata* L.) on the physiological parameters of purple nutsedge (*Cyperus rotundus* L.) at 40 DAP.

Parameters	Treatments				
	C−	C+	C10	C20	C40
Chlorophyll a (mg g^{-1} FW)	0.63 ± 0.004 a	0.63 ± 0.001 a	0.53 ± 0.003 b	0.50 ± 0.005 c	0.43 ± 0.003 d
Chlorophyll b (mg g^{-1} FW)	1.13 ± 0.007 a	1.13 ± 0.003 a	0.95 ± 0.005 b	0.92 ± 0.008 c	0.80 ± 0.005 d
Total chlorophyll (mg g^{-1} FW)	1.05 ± 0.004 a	1.02 ± 0.007 b	0.76 ± 0.005 c	0.70 ± 0.003 d	0.60 ± 0.006 e
Carotenoid (mg g^{-1} FW)	0.29 ± 0.0003 a	0.27 ± 0.0029 b	0.20 ± 0.0036 c	0.19 ± 0.0013 c	0.17 ± 0.0017 d

Mean values followed by the same letters are not significantly different at $p = 0.05$ (ANOVA and Tukey's test) and $n = 4$. The error bars indicate the standard error. C− = aquadest; C+ = gallic acid; C10 = 10% wedelia extract concentration; C20 = 20% wedelia extract concentration; and C40 = 40% wedelia extract concentration.

3.4. Biochemical Parameters

3.4.1. Lipid Peroxidation (MDA)

The impact of the wedelia leaf extract on the lipid peroxidation activity of purple nutsedge at 40 DAP was significant. The level of lipid peroxidation was assessed by measuring the MDA content. A gradual increase in the MDA content was recorded in treated plants; however, the amount of MDA content depended on the wedelia extract concentration. The lowest MDA content was recorded in the control group (Table 5).

3.4.2. Hydrogen Peroxide

The effect that the wedelia leaf extract had on hydrogen peroxide significantly differed from that of the control treatment at 40 DAP. An increase in hydrogen peroxide content was recorded in treated plants but was dependent upon wedelia extract concentration. The highest content of hydrogen peroxide was found at concentrations of 40% (Table 5).

Table 5. Effect of wedelia extract (*Wedelia trilobata* L.) on the biochemical parameters of purple nutsedge (*Cyperus rotundus* L.) at 40 DAP.

Parameters	Treatments				
	C−	C+	C10	C20	C40
Malondialdehyde (nmol g^{-1} FW)	14.20 ± 0.704 b	14.36 ± 0.276 b	19.87 ± 0.496 a	21.18 ± 0.918 a	21.27 ± 0.650 a
Hydrogen peroxide (mol g^{-1} FW)	19.29 ± 6.402 d	28.74 ± 4.275 cd	39.62 ± 7.237 bc	48.40 ± 6.830 b	68.64 ± 3.841 a
Peroxidase (U min^{-1} g^{-1} FW)	0.006 ± 0.0025 a	0.003 ± 0.0005 ab	0.002 ± 0.0008 ab	0.002 ± 0.0008 b	0.001 ± 0.0005 b

Mean values followed by the same letters are not significantly different at $p = 0.05$ (ANOVA and Tukey's test) and $n = 4$. The error bars indicate the standard error. C− = aquadest; C+ = gallic acid; C10 = 10% wedelia extract concentration; C20 = 20% wedelia extract concentration; and C40 = 40% wedelia extract concentration.

3.4.3. Peroxide

Maximum POD activity was recorded in plants treated with a lower concentration of wedelia extract. The highest concentration of wedelia leaf extract (40%) significantly suppressed POD activity (Table 5).

3.5. Tuber Anatomy

In transverse of purple nutsedge rhizome, vascular bundles (VBs) are distributed around the perimeter of central pith and shown a significantly different number of VBs. The number of VBs approximately found 33 in control, decreased to 13 in C+, 17 in C10%, 27 in C20%, and 24 in C40% (Table 2 and Figure 4). Wedelia leaf extract application also affected the appearance of starch. The highest concentration of wedelia leaf extract (40%) had no starch formation (Figure 4).

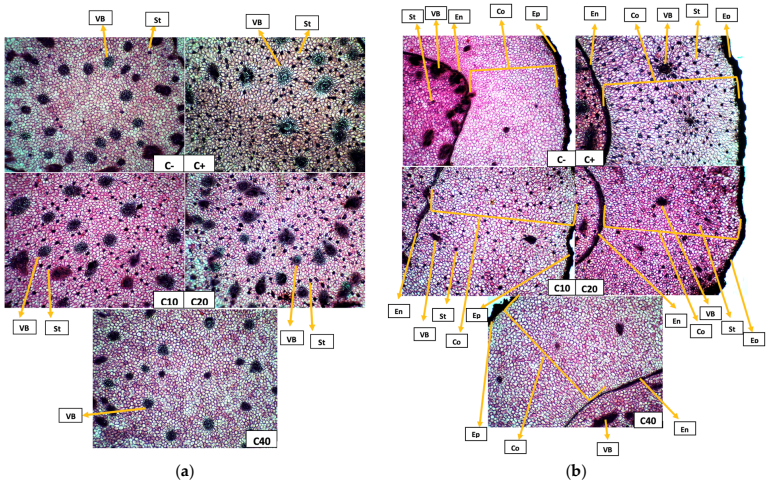


Figure 4. Transverse section of mother tubers of purple nutsedge (*Cyperus rotundus* L.) at 40 DAP. VB = vascular bundles; En = endodermis; Ep = epidermis; Co = cortex; St = starch; C− = aquadest; C+ = gallic acid; C10 = 10% extract (*Wedelia trilobata* L.) extract concentration; C20 = 20% wedelia extract concentration; and C40 = 40% wedelia extract concentration. (**a**) transverse section of the cortex from mother tubers of purple nutsedge; (**b**) transverse section showing both the epidermis and endodermis from mother tubers of purple nutsedge.

3.6. Correlation between Parameters

Our study revealed that there is a correlation between parameters. The total chlorophyll was positively correlated with the fresh and dry plant weights and showed a significant difference at $p < 0.05$. Carotenoids were positively correlated with the fresh and dry plant weights and showed significant differences at $p < 0.01$ and $p < 0.05$, respectively. MDA levels were positively correlated with H_2O_2 levels and showed a significant difference at

$p < 0.001$. Peroxidase levels were negatively correlated with MDA and H_2O_2 levels and were significantly different at $p < 0.001$ and $p < 0.01$, respectively (Figure 5).

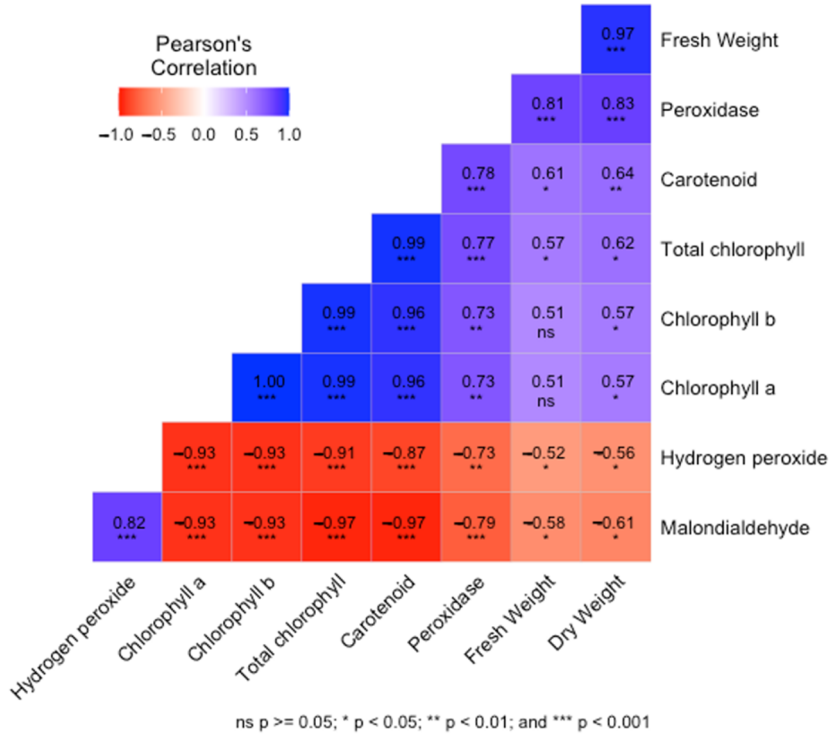


Figure 5. Correlation between parameters due to application of wedelia leaf (*Wedelia trilobata* L.) extract at 40 DAP in purple nutsedge (*Cyperus rotundus* L.).

4. Discussion

Purple nutsedge, a persistent and invasive weed, poses a significant challenge for farmers worldwide. Excessive quantities of synthetic herbicides are being employed globally to combat its rapid spread and harmful effects on crop yields; however, the use of these chemicals raises concerns due to their negative impact on the environment and human health. In recognition of these issues, agricultural researchers and scientists are actively working to discover alternative, eco-friendly weed control methods. One promising approach is the use of natural herbicides derived from plant extracts or microorganisms.

Hans Molisch (1937) identified the phenomenon of allelopathy at the beginning of the 20th century as the impact of one plant on another through the release of chemicals into the environment [34]. Allelopathy involves secondary metabolites, termed allelochemicals [35], produced by plants, viruses, fungi, and microorganisms that influence the development and growth of crop production and ecosystems (including animals) and has advantages and disadvantages. Allelochemicals are released into nature as plant tissue decomposition exudates, volatiles, and/or residues [36] and affect plant structure at the molecular, structural, biochemical, physiological, and ecological levels. Allelopathic compounds can cause secondary oxidative stress, which presents as increased reactive oxygen species (ROS) generation, increased free radical production, and the induction of the cellular antioxidant system [37].

ROS are well-known signaling compounds that control plant responses to both abiotic and biotic stresses [38]. Plants produce ROS in response to environmental stresses such as high or low temperature, high light, drought, salinity, nutrient deficiency, and pathogen infections. Oxidative stress occurs when there is an imbalance between ROS production and detoxification through enzymatic and nonenzymatic reactions. Increased net ROS formation triggers photooxidative damage to DNA, lipids, and proteins, which ultimately leads to cell death [39].

Under abiotic and biotic stress, aerobic metabolism, high-energy exhibition, and electron-transfer reactions reduce molecular oxygen (O_2), in a stepwise manner and results in the formation of highly reactive ROS, such as singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO·) [39]. In this study, the wedelia leaf extract treatments caused plant stress, as indicated by the increased levels of hydrogen peroxide (H_2O_2) contents in purple nutsedge, which increased as the extract concentration level increased. These results are similar to those of a previous study, in which allelochemical stress-induced oxidative damage in lettuce via an increased production of hydrogen peroxide (H_2O_2) [40].

ROS accumulation induces membrane lipid peroxidation and electrolyte release from the cells [41]. The MDA concentrations in the purple nutsedge were used to determine lipid peroxidation [42]. Specifically, the wedelia leaf extract increased the MDA content of the plant in response to allelochemical stress, which resulted in oxidative stress due to ROS formation. Previously, oxidative stress was also indicated by an increased MDA level in *Cyperus roundus* as a reaction to the allelochemicals of sesame plant leachate [7]. Allelochemical stress (i.e., lipid peroxidation) was also indicated in lettuce root due to increased MDA contents [40].

Plants have evolved two types of functionally interconnected oxidative stress defense mechanisms: enzymatic and non-enzymatic defense [38]. The enzymatic components include peroxide (POD), superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), glutathione-S-transferase (GST), and catalase (CAT), and the non-enzymatic include low molecular compounds, such as ascorbic acid (AA), reduced glutathione (GSH), carotenoids, α-tocopherol, flavonoids, phenolics, and proline [43–45]. In this study, the allelochemicals from the wedelia leaf extract decreased the POD content in purple nutsedge. Decreased enzyme activity at the highest concentration of wedelia leaf extract indicated that the plant failed to form an enzymatic detoxification defense mechanism. This is consistent with the findings of a previous study that found that plants treated with lower concentrations of leachate had higher SOD activities, whereas plants treated with the highest concentration of leachate (100%) had lower SOD activities. At the highest leachate concentration, SOD enzyme activity was reduced, indicating a possible antioxidant defense failure [7]. The consistently low level of POD in the treated plant suggests that the antioxidant enzyme may not be completely effective in eliminating ROS. According to the findings of [46], the allelochemicals in wedelia leaf extract may directly inhibit oxidizing enzymes, to thus make the plant vulnerable to oxidative damage.

Carotenoids are plant pigments that play a crucial role in photosynthesis and protect against oxidative stress caused by reactive oxygen species (ROS). Plants have developed various defense mechanisms to cope with ROS, and carotenoids are one such defense system. The activity of the electron transport chain (ETC) may be insufficient to protect against photo-oxidative damage caused by a decrease in carotenoid contents. Key enzymes in carotenoid biosynthesis that are inhibited by secondary metabolites include 4-hydroxyphenylpyruate dioxygenase and/or phytoene desaturase [47]. The carotenoid contents decreased significantly in this study when treated with wedelia leaf extract. This is consistent with the findings of a previous studies, which found that the carotenoid contents decreased significantly in the presence of sesame leachate [7] and in treated plants compared to controls [42].

Chloroplasts are particularly susceptible to oxidative damage. ROS can directly damage chlorophyll molecules through oxidation. This damage disrupts the structure and function of chlorophyll, resulting in reduced chlorophyll contents. Furthermore, high ROS levels can activate enzymes involved in chlorophyll degradation, such as chlorophyllase, which accelerates the breakdown of chlorophyll molecules and further reduces chlorophyll contents [48,49]. In this study, wedelia leaf extract had a negative effect on the pigment contents of purple nutsedge. Specifically, the allelochemicals inhibited the amount of chlorophyll a, chlorophyll b, and total chlorophyll present and had an adverse effect on plant growth. Similar reductions in the chlorophyll a, chlorophyll b, and total chlorophyll

contents have been reported in many other plants after treatment with different weed extracts. Chlorophyll suppression can occur as a result of impeding either the biosynthesis or degradation of chlorophyll components, which can be influenced by allelochemicals. The reduction of chlorophyll content leads to a decline in the photosynthetic rate and the controlled accumulation of photosynthates. Consequently, plants resort to using their food reserves, which results in stunted plant growth and the proliferation of rhizomes and tubers [7]. In a previous study, wedelia leaf aqueous extract significantly reduced the leaf chlorophyll content of rice by 40% compared to the control treatment [24]. Similarly, chlorophyll contents were reduced in all plants treated with aqueous extracts made from various parts of *Ageratum conyzoides*, but the greatest reduction was observed in plants treated with leaf extract during all stages of mungbean growth. *Ageratum conyzoides* allelochemicals reduced chlorophyll content in mungbean plants by inhibiting biosynthesis or increasing degradation [28]. The production of chlorophyll was also suppressed by the allelopathic activity of mangiferin in radish seedlings [50].

During the photosynthesis process, chlorophyll functions as a photocatalyst. When it is in its energized state, which results from the absorption of light, it catalyzes an energy-storing chemical reaction. There are two chlorophylls present in green plants: chlorophyll a and chlorophyll b [51]. These molecules are crucial to photosynthesis as the primary pigments found in leaves and facilitate the absorption of solar radiation. This process serves as the initial stage of the photosynthetic pathway [52]. Light is absorbed by the antenna pigments and transferred to the reaction centers of PSI and PSII during photosynthesis. These centers initiate primary photochemical reactions, which convert light energy into chemical energy with little dissipation. The main source of dissipation is chlorophyll fluorescence from PSII-associated molecules. Allelochemical stress is frequently the first to inhibit photosynthetic processes, which are typically the first to be influenced by allelochemicals [53–55]. The reduction in chlorophyll contents indicate that the cells' ability to synthesize chlorophyll had decreased [56]. There is also evidence of a reduction in the antenna size of photosynthetic reaction center complexes [57].

Reduced chlorophyll impairs the efficiency of the photosynthetic process, which may lead plants to redirect energy and resources away from growth processes and results in growth inhibition. The energy that would have been used for growth is redirected toward repairing cellular damage caused by oxidative stress [48,49]. There is much evidence to show that wedelia inhibits the growth of other plants through chemicals produced by its parts. Our experiment shows that growth decreased with increasing wedelia leaf extract concentrations. The allelochemicals present in wedelia leaf extract may inhibit or cause the cessation of cell division by inhibiting or causing the expression and elongation of bud cells [7]. Furthermore, fresh wedelia leaf extract had a significant effect on the shoot and root length of *C. arietinum*, *V. unguiculata*, and *V. radiata* seedlings and significantly reduced their fresh and dry weights. The highest inhibition occurred at the highest concentration (75%), while the lowest inhibition was found at the lowest concentration (25%). Khan (2021) [58] discovered that the germination reduction trend (%) corresponded to the applied extract concentrations, indicating that higher inhibitory effects were found at higher concentrations and lower at lower concentrations. Shoot and root lengths were found to decrease as treatment concentrations increased. The higher inhibitory effect due to increasing concentration was also found to have an effect when administering *Couroupita guianensis* Aubl extract. Both seeds' inhibitions of germination were related to extract concentrations, indicating that inhibition of germination increased with increasing extract concentrations [59]. The treated plants' fresh and dry weights were significantly reduced, which could be attributed to a decrease in the metabolic activities of the aerial and underground plant parts [23]. Additionally, wedelia aqueous extract was found to inhibit the growth of rice seedlings. Plants treated with aqueous extracts of wedelia root, stem, leaf, and whole plant had 45.45%, 19.09%, 25.45%, and 34.55% of the control tillers, respectively [24]. Sesame leachate at the highest concentration (100%) caused a decrease in the biomass of *Cyperus rotundus* by 82% and 83% at 20 DAS and 40 DAS, respectively [7], the leaf extracts of *Tephrosia purpurea*,

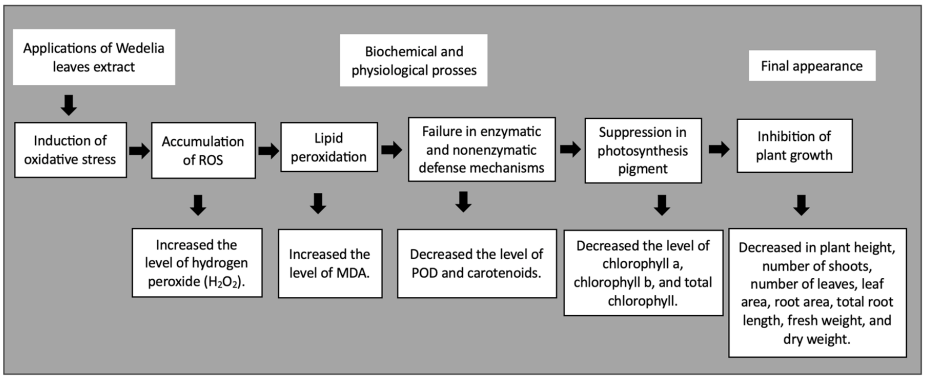
Albizia amara, and *Delonix regia* reduced maize biomass [60], and the wedelia leaves aqueous extracts reduced 81% dry weight on rice compared to the control treatment [24].

Plant stress, including oxidative stress, can have systemic effects on various aspects of Plant stress, including oxidative stress, can have systemic effects on various aspects of plant growth and development, including vascular tissue formation. Stress conditions may lead to the redistribution of resources within the plant. Prioritizing resources for stress response may affect the allocation of nutrients and energy needed for the development of vascular bundles. Stress-induced changes in hormonal balance may have an indirect effect on vascular tissue formation. Vascular bundles are underdeveloped and abortive because the number, size, and amount of phloem and xylem are reduced [7].

5. Conclusions

The allelopathic potential of wedelia leaf extract against purple nutsedge is attributed to the induction of oxidative stress, as evidenced by the accumulation of ROS, particularly hydrogen peroxide. This oxidative stress triggers lipid peroxidation, as indicated by an increase in MDA levels. The consequences of lipid peroxidation extend to both enzymatic and nonenzymatic defense mechanisms. First, the enzymatic defense mechanism is compromised, with a notable decrease in POD activities. This reduction suggests a failure in the plant's ability to counteract oxidative stress via enzymatic means. Simultaneously, the nonenzymatic defense mechanism, specifically carotenoids, is also impacted, further diminishing the plant's capacity to combat oxidative damage. The cumulative effect of these biochemical changes leads to the suppression of photosynthetic pigments, primarily chlorophyll. This suppression directly hampers the photosynthetic process, which affects the overall growth and development of purple nutsedge. The observed inhibition manifests as reduced plant height, a decrease in the number of shoots and leaves, diminished leaf area, compromised root area, and a decline in both total root length and biomass, including fresh and dry weights.

The cascade of events initiated by the allelochemicals in wedelia leaf extract creates a hostile environment for purple nutsedge by disrupting its antioxidative defense mechanisms, compromising photosynthetic pigments, and ultimately stunting its growth. This comprehensive understanding of the allelopathic effects provides valuable insights for the development of sustainable weed management strategies, highlighting the potential of Wedelia leaf extract as an effective bioherbicide against purple nutsedge (Scheme 2).



Scheme 2. The biochemical and physiological mechanisms of wedelia leaf extract in inhibiting the growth of purple nutsedge.

Author Contributions: Conceptualization, D.W.R. and Q.U.; methodology, D.W.R. and Q.U.; software, Q.U.; validation, Q.U. and D.I.; formal analysis, Q.U.; investigation, Q.U. and D.I.; resources, Q.U.; data curation, Q.U.; writing—original draft preparation, Q.U.; writing—review and editing, Q.U.; visualization, Q.U.; supervision, D.W.R. and D.I.; project administration, D.W.R. and D.I.; funding acquisition, D.W.R. and D.I. All authors have read and agreed to the published version of the manuscript.

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