Plant Soil (2025) 511:163–187
https://doi.org/10.1007/s11104-024-06979-y

RESEARCH ARTICLE

Elucidating the interactive effects of drought, weeds, and herbicides on the physiological, biochemical, and yield characteristics of rice

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Received: 27 April 2024 / Accepted: 22 September 2024 / Published online: 1 October 2024

Abstract
Aims Rice yields are significantly influenced by both biotic and abiotic factors, like drought stress and weed infestation being prominent contributors to substantial crop losses. Environmental conditions, including drought stress, can impact the effectiveness of herbicides. This study aims to investigate the impact of drought stress on the efficacy of the herbicide (cyhalofop + penoxsulam) against the weed species, *Echinochloa colona* (L.) Link, and *Alternanthera paronychioides* A. St.-Hil. Additionally, the study aims to assess the potential consequences of improper weed control, including the survival of weeds and their subsequent adverse effects on rice.
Methods The herbicide was applied to rice plants under two distinct conditions: well-watered (WW) and drought-stressed (DS) at 8 days following the suspension of irrigation. The herbicide's effectiveness against two weed species, *E. colona* and *A. paronychioides*, was assessed by quantifying weed growth and biomass.

Responsible Editor: Al Imran Malik.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06979-y.

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Results The findings elucidate a reduction in the herbicide efficacy against both the weeds under DS conditions. However, under DS the decline in herbicide effectiveness was more significant against *E. colona* than *A. paronychioides*, leading to inadequate weed control. As a result, the survival of these weeds further exacerbates oxidative stress in rice plants. The magnitude of oxidative stress was excess in rice with *E. colona* than *A. paronychioides*, and it significantly reduced the yield under both WW and DS.

Conclusions The study highlighted that under drought conditions rice is more susceptible to *E. colona* infestation than to *A. paronychioides* with increased oxidative stress and reduced yield. The study underscores the critical importance of understanding complex interactions between multiple environmental factors in developing adaptive, climate-resilient weed management strategies, essential for safeguarding rice yields against the combined impacts of drought stress and weed interference, and addressing climate change-related challenges to food security.

Keywords Drought stress · Rice · *A. paronychioides* · *E. Colona* · Herbicide efficacy · Cyhalofop + penoxsulam

Introduction

Rice (*Oryza sativa* L.) stands as a crucial global food crop, responsible for nourishing nearly half of the world's population. In India alone, rice cultivation encompasses an extensive area of approximately 43.8 million hectares, yielding around 117.5 million tons (Directorate of Economics and Statistics 2019). On a global scale, the year 2019 witnessed rice production reaching an impressive 750 million tons (FAO 2019). However, as we look ahead to a projected global population of 9.7 billion by 2050 (World population prospects 2019), the imperative of doubling current agricultural output becomes apparent to meet the burgeoning demand (Noureldin et al. 2013). Despite this, the average annual increase in rice yields hovers at a modest 1%, insufficient to attain the targeted levels by 2050 (Ray et al. 2013).

The path toward higher rice productivity is obstructed by an array of abiotic and biotic stresses, acting as prominent barriers (Bajwa et al. 2015; Zafar et al. 2017; Oliva et al. 2019; Ahmed et al. 2021). Rice, a water-dependent crop, is extremely susceptible to both drought stress and weed infestations (El-Metwally and Saudy 2021b; Saudy et al. 2021c, 2022). These stresses induce the synthesis of Reactive Oxygen Species (ROS) (Abdelaal et al. 2022; Sreekanth et al. 2024) encompassing species such as H_2O_2, hydroxyl radicals (OH·), O_2·-, and singlet oxygen (O_2) (Li et al. 2022). When ROS production surpasses thresholds, oxidative stress within cells ensues, leading to damage in lipids, carbohydrates, proteins, and nucleic acids, ultimately culminating in cell death (Saad-Allah et al. 2021). This phenomenon detrimentally affects various physiological and biochemical traits of crops, encompassing water intake, carbon assimilation, relative water content, electrolyte leakage, antioxidant compounds, and yield (Saudy 2015; Mubarak et al. 2021; Salem et al. 2021; Abdelaal et al. 2022).

Drought stress can hinder the growth, maturation, and reproductive processes of both crops and weeds (Ziska et al. 2004; Chahal et al. 2018). Nonetheless, specific weeds like *Hordeum murinum* L., *Bromus tectorum* L., and *Lactuca serriola* L. demonstrate heightened adaptability to such climatic factors because of phenotypic versatility (Jabran and Dogan 2018). The challenge of crop production is anticipated to intensify due to well-adapted weeds in a climate-change situation with high CO_2 and water scarcity (Ziska 2016).

In most cases, it is commonly acknowledged that during periods of drought, the competition between weeds and crops for water intensifies, often benefiting certain weed species (Patterson 1995). Drought-induced modifications in crop physiology, biochemistry, and molecular responses often impede the movement and uptake of herbicides, ultimately limiting their efficacy and promoting weed survival (Miller and Norsworthy 2018; Skelton et al. 2016; Sreekanth et al. 2023; Wu et al. 2019). The advent of climate change adds another layer of complexity to the interaction between environmental factors and herbicide efficiency, influenced by variables like soil moisture, relative humidity, light, and temperature (Varanasi et al. 2016). Earlier investigations revealed the decreased effectiveness of certain herbicides, like fenoxaprop, under drought (Xie et al. 1993). Xie et al. (1996) stated a neutral effect of drought on some herbicides, like imazamethabenz. Assessing the efficacy of herbicides on weed species under drought stress is necessary. Additionally, if there is a reduction in herbicide performance, it is required to estimate the degree of the reduction and adjust the herbicide rates to achieve effective weed control. Thus, understanding the impact of climate change variables, such as drought stress, on weed growth and the efficacy of herbicides is crucial for efficient weed management (Varanasi et al. 2016).

For efficient weed management and maximal crop productivity, understanding the interplay of factors such as drought stress and herbicide efficacy is indispensable (Heap and Duke 2018; Heap 2014; Matzrafi et al. 2019). Notably, drought stress can impede herbicide efficacy, particularly affecting the weeds response to herbicides and limiting the active ingredient's ability to reach its intended site (Weller et al. 2019).

E.colona and *A. paronychioides* infestation in rice fields can result in significant reductions in grain yield, between 30 and 100% (Rao et al. 2007; Chauhan and Johnson 2010) and 62–83% (Pawar et al. 2022), respectively. Several investigations have explored the reactions of numerous weed species to water stress (Matloob et al. 2015), as well as the physiological responses of rice to drought (Abdelaal et al. 2022).

Knowledge about the influence of drought on weeds and the efficacy of herbicides is crucial for optimizing the effectiveness of herbicide applications (Alizade et al. 2021). It was hypothesized that the drought stress lowers the efficacy of the herbicide (cyhalofop + penoxsulam) against the targeted weeds, *A. paronychioides* and *E. colona*. This decrease in herbicide efficacy results in improper control of weeds, and the weeds that do survive due to reduced herbicide efficacy are likely to adversely affect rice physiological, biochemical traits and yield attributes. Therefore, considering these facts and the growing menace of *A. paronychioides* and *E. colona* in rice production, a study was carried out with rice (Sahbhagi) solely and rice with its predominant weeds *Echinochloa colona* (L.) Link. and *Alternanthera paronychioides* A. St.-Hil. along with ready-mix post-emergence herbicide (cyhalofop + penoxsulam) under drought with the following objective: The current study aims to explore the impact of drought stress on the efficacy of the herbicide (cyhalofop + penoxsulam) against the targeted weed species, *A. paronychioides* and *E. colona*. Moreover, the investigation aims to assess the probable consequences of improper weed control, including the survival of weeds and their subsequent adverse effects on rice physiological, biochemical traits, and yield attributes. This research reinforces the importance of understanding the intertwined influences of biotic and abiotic stressors in shaping agricultural outcomes. The identified correlations between drought stress, herbicide efficacy, weed growth, and rice physiology provide a stepping stone for future endeavors aimed at fortifying agricultural systems against the challenges posed by these stresses.

The study investigates the following research question: How does drought stress impact the efficacy of the herbicide (cyhalofop + penoxsulam) against the targeted weeds, *A. paronychioides* and *E. colona*, and what are the potential effects of surviving weeds on rice physiological, biochemical traits, and yield attributes under drought conditions.

Materials and methods

A pot experiment was conducted during *Kharif*-2022 and 2023 in a green-house at the Indian Council of Agricultural Research - Directorate of Weed Research (ICAR-DWR), Jabalpur, Madhya Pradesh, India, utilizing rice variety Sahbhagi and its primary weeds, *Alternanthera paronychioides* and *Echinochloa colona*. The green-house was carefully regulated to ensure controlled conditions (12 h light, 28 ± 2°C temperature, 60–75% RH and light intensity of 500–1000 µmol photons m^{-2} s^{-1}). The soil exhibits a clay loam texture, possesses a low organic carbon matter, and has a pH level of 7.6. The study was carried out using rice alone and rice grown with its primary weeds, *A. paronychioides* and *E. colona*. The plants were grown in earthen pots (42 cm height X 46 cm diameter) filled with 58 kg soil. Initially, we broadcasted 20 seeds of each weed species along with rice seeds. 7 to 8 after weed germination, they were maintained at 25/m^2 and 50/m^2 of *A. paronychioides* and *E. colona*, respectively by uprooting the excess weeds. Weed-free rice pots were kept as 'weed-free' by regularly uprooting the emerged weeds after every 5–7 days interval (Table 1). Fertilizers were administered at the recommended rate (120:60:40 N, P_2O_5, K_2O Kg/ha) to all experimental pots. Phosphorus (P) and potassium (K) fertilizers were used as a basal

Table 1 Experimental treatments details

Treatments		Description
Control(Well-watered, WW)	WW-RWF	Well-watered Weed free rice
	WW-RA	Well-watered Rice + *A. paronychioides*
	WW-RAH	Well-watered Rice + *A. paronychioides* + Herbicide
	WW-RE	Well-watered Rice + *E. colona*
	WW-REH	Well-watered Rice + *E. colona* + Herbicide
Drought(Drought stress, DS)	DS-RWF	Drought-stressed Weed free rice
	DS-RA	Drought-stressed Rice + *A. paronychioides*
	DS-RAH	Drought-stressed Rice + *A. paronychioides* + Herbicide
	DS-RE	Drought-stressed Rice + *E. colona*
	DS-REH	Drought-stressed Rice + *E. colona* + Herbicide

dose, while nitrogen (N) fertilizer was distributed in three distinct stages: 50% at sowing, 25% during active tillering, and the remaining 25% at the booting stage (Bahuguna et al. 2015). Experimental pots were organized randomly as per completely randomized design (CRD) with each treatment having five replications (i.e., five pots per treatment).

Drought imposition and application of herbicide

Well-watered (WW) pots were frequently watered, while drought stress (DS) was initiated by refraining from irrigation starting at 25 days after sowing (DAS) for a duration of 15 days. A post-emergence herbicide ready mix [cyhalofop-buty + penoxsulam (135 g ai/ha^{-1})] was employed to WW and DS pots at 8 days after irrigation was withheld. The herbicide, cyhalofop-Butyl 5.1% + penoxsulam 1.02% OD (manufactured by Dow Agro Sciences), was applied using a flat fan nozzle sprayer at a volume of 375 L per hectare, with water as the spray solution. The plants were rewatered after noticing drought indications like leaf yellowness, drying, and leaf rolling.

Soil moisture content (SMC)

Soil samples from both the top 5 cm and bottom 5 cm of each pot were collected in triplicate. These samples were obtained using sampling auger equipment and placed into pre-weighed boxes. To assess their fresh weight, soil samples of all treatments were weighed initially. Subsequently, these samples were dried for up to one day in an oven set to 105°–110 °C. Soil moisture content (SMC) was then determined according to Schmugge et al. (1980):

$$\mathbf{SMC} = \left[\text{Weight of wet soil} - \text{Weight of dry soil} / \text{Weight of wet soil}\right] \times 100$$

Assessment of physiological and biochemical indices

Before to rewatering (45 DAS), relative water content (RWC), membrane stability index (MSI), and gas exchange traits were determined. Leaf samples were aseptically collected for biochemical analysis, RWC and MSI.

Relative water content (RWC)

Fresh leaf samples (FW) were submerged in Petri plates filled with double distilled water for 4 h to record the turgid weight (TW). After overnight oven drying at 70 °C, the dry weight (DW) was measured, and RWC was estimated from Barrs and Weatherley (1962) equation.

$$\mathbf{RWC}\ (\%) = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

Membrane stability index (MSI)

The MSI of the fresh leaves was assessed using the protocol proposed by Sairam (1994). The MSI was assessed as:

$$\mathbf{MSI} = \left[1 - (\text{C1}/\text{C2})\right] \times 100$$

Chlorophyll-a and chlorophyll-b content

A 0.1 g leaf sample was incubated in 10 mL of acetone (80%). Absorbance was noted at 663 nm and 645 nm (Lichtenthaler 1987).

Measurement of gas exchange parameters

The LI-COR portable photosynthesis system was utilized to record the net photosynthesis rate, stomatal conductance, and transpiration. These indices were assessed from the flag leaf between 10:00–12:00 h (Leakey et al. 2004).

Oxidative stress markers

Superoxide anion content (o2.$^-$)

The $O_2.^-$ level was estimated by measuring absorbance at 530 nm with sodium nitrite standard and specified as μmol g^{-1} FW (Wu et al. 2010).

Hydrogen peroxide content (H_2O_2)

H_2O_2 levels was determined using Mukherjee and Choudhari's (1983) protocol, recording the absorbance at 415 nm and quantifying it with an H_2O_2 standard curve, mentioned as μmol g^{-1} FW.

Malondialdehyde (MDA) analysis

The MDA was assessed using an extinction coefficient of 155 mM^{-1} cm^{-1} by taking absorbance at 532 and 600 nm and specified as μg g^{-1} FW (Heath and Packers 1968).

Antioxidative enzymes assay

A 1 g leaf sample was ground in an extraction solution containing 0.1 M phosphate buffer, 0.5 mM EDTA, 1% polyvinyl pyrrolidone, and 1 mM PMSF. After centrifugation at 12,000 g for 30 min at 4 °C, the resulting supernatant was utilized to evaluate SOD, CAT, and POD enzymatic activities. The quantification of protein levels was done by Lowry et al. (1951) method.

Assessment of SOD (EC 1.15.1.1) activity was done by measuring absorbance at 560 nm and measured in units of mg $protein^{-1}$ min^{-1} (Beauchamp and Fridovich 1971).

The activity of CAT (E.C. 1.11.1.6) was done using an extinction coefficient of 45.2 mM^{-1} cm^{-1} by measuring the consumption of H_2O_2 at 240 nm for 1 min (Aebi 1974).

Peroxidase (POD, EC 1.11.1.7) activity was examined following the protocol proposed by Kar and Mishra (1976), tracking purpurogallin generation at 420 nm using a blank.

Non-enzymatic antioxidants

Proline content

Quantification of proline was done by measuring absorbance at 520 nm (Bates et al. 1973).

Total phenolic content (TPC)

The TPC was evaluated by the methodology of Singleton and Rossi (1965) by measuring the OD at 765 nm.

Ascorbic acid content (asa)

AsA was assessed following the Hodges et al. (1996) procedure, with absorbance recorded at 525 nm. AsA content was determined using the L-ascorbic acid standard curve.

Starch content

The leaf's starch content was investigated by the methodology given by McCready et al. (1950). Liquid nitrogen was used to grind the leaf samples. 0.1 g of the sample was mixed with 80% ethanol (V/V) and then centrifuged. The pellet obtained after centrifugation was dried, and perchloric acid was supplemented to extract starch from the sample. This mixture was centrifuged again and then analyzed using an anthrone reagent.

Rice grain yield

Grain yield at maturity (108 DAS) was estimated by randomly sampling five plants from each treatment.

Weed growth and biomass traits

Plant fresh weight, dry weight, leaf area and root length of weeds were recorded at 7 days after herbicide application. The herbicide application was conducted 8 days after irrigation was withheld, which corresponds to 33 days after sowing (DAS).

Statistical analysis

Analysis of variance (ANOVA) was used to assess the main effects and interactions of multiple independent factors on physiological parameters, as it fits the experimental design. Significant differences identified by ANOVA were further explored using Tukey's HSD test, which provided pairwise comparisons between treatment groups while controlling for Type I error, ensuring accurate identification and reporting of significant differences between various treatments and weed control strategies. The ANOVA was assessed by SPSS Inc., Chicago, IL, USA (Version 16.0). A post-hoc test (Tukey's) was carried out to determine the differences between treatments with a least significant difference (LSD) of 5% and 1%. The correlation and PCA analysis were performed using Minitab.

Results

Soil moisture content (SMC)

SMC significantly varied across treatments. In weed-free rice under well-watered (WW) conditions, SMC measured 21.60% (at top 5 cm) and 22.56% (below 5 cm soil). However, under drought stress (DS), SMC decreased to 10.51% (top 5 cm soil) and 11.55% (below 5 cm soil). With the interference of *A. paronychioides*, SMC dropped to 17.15% (top 5 cm soil) and 18.24% (below 5 cm soil) in WW conditions, and even further to 8.86% (top 5 cm soil) and 9.87% (below 5 cm soil) in DS conditions. In contrast, *E. colona* interference reduced SMC to 16.90% (top 5 cm) and 17.85% (below 5 cm soil) in WW conditions, and drastically to 6.70% (top 5 cm) and 7.61% (below 5 cm soil) in DS conditions. Similar SMC trends were observed in herbicide-treated pots. *E. colona* presence during DS led to a significant SMC reduction, suggesting extreme drought stress on rice by *E. colona* (see Supplementary Fig. 1a & b).

Physiological and biochemical traits

Relative water content

A significant variability across Weed, Crop and Herbicide (WCH) (Rice alone; rice + weeds; rice + weeds + herbicide) ($P < 0.001$), T (Control and drought) ($P < 0.001$) and WCH X T ($P < 0.001$) for RWC was noticed. The RWC values under WW and DS conditions in weed-free rice were 86.74% and 71.60%, respectively (Table 2). In both WW and DS circumstances, weed interference (*A. paronychioides* and *E. colona*) substantially lowered the RWC. The presence of *A. paronychioides* led to a notable decrease in RWC by 15.16% and 18.95% in WW and DS, respectively, compared to the weed-free control. Likewise, *E. colona* interference resulted in RWC reductions by 22.17% and 24.45% under WW and DS, respectively. This highlights severe drought stress experienced by rice due to both weed species in both WW and DS conditions (Fig. 1; Supplementary Fig. 2a and b). The application of herbicide resulted in decreased weed stress on rice plants, leading to a remarkable increase in the RWC of rice grown with *A. paronychioides* (12.97% and 13.71% during WW and DS conditions, respectively) and *E. colona* (6.38% and 3.32% during WW and DS, respectively), compared to their respective herbicide unsprayed controls (Table 2).

Membrane stability index

There was a noteworthy variation seen for MSI among WCH ($P < 0.001$), T ($P < 0.001$), and WCH X T ($P < 0.05$). The MSI values for weed-free rice were 82.64% and 77.63% in WW and DS, respectively (Table 2). *A. paronychioides* presence led to MSI reductions of 16.05% and 23.45% in WW and DS compared to the weed-free control. Conversely, *E. colona* caused MSI decreases to 22.86% and 25.18% in WW and DS relative to the control. Rice grown with *A. paronychioides* (11.06% and 21.35% at WW and DS, respectively) and *E. colona* (20.14%% and 10.40% at WW and DS, respectively) had significantly higher MSI than their respective

Table 2 Effects of drought and weed management on the RWC, MSI, and chlorophyll a and chlorophyll b levels of rice plants

Weed, Crop and Herbicide (WCH)	Treatment (T)	RWC (%)	MSI (%)	Chlorophyll-a content (mg g^{-1} FW)	Chlorophyll-b content (mg g^{-1} FW)
RWF	WW	84.21 ± 0.82a	82.64 ± 0.33a	4.24 ± 0.12a	2.03 ± 0.16a
	WDS	74.25 ± 0.61d	77.63 ± 0.83b	2.55 ± 0.25b	0.98 ± 0.14b
RA	WW	71.44 ± 0.49e	69.37 ± 0.78c	2.01 ± 0.11c	0.85 ± 0.11c
	WDS	68.26 ± 0.69f	63.26 ± 1.41e	2.61 ± 0.17c	1.12 ± 0.19c
RAH	WW	80.70 ± 0.67b	77.05 ± 0.82b	2.65 ± 0.22d	1.15 ± 0.21d
	WDS	77.62 ± 0.86c	76.76 ± 4.02b	3.16 ± 0.16e	1.51 ± 0.04e
RE	WW	65.54 ± 0.10g	63.74 ± 0.52de	1.70 ± 0.11f	0.63 ± 0.16e
	WDS	63.62 ± 0.36h	61.83 ± 1.40e	1.32 ± 0.18g	0.55 ± 0.11f
REH	WW	69.72 ± 0.69ef	76.58 ± 0.86b	2.19 ± 0.14h	0.88 ± 0.16fg
	WDS	65.74 ± 0.33g	68.26 ± 1.04cd	1.39 ± 0.12i	0.59 ± 0.14g
	WC	0.806***	2.07***	0.018***	0.006***
Lsd < 0.05	T	1.274***	3.273***	0.028***	0.010***
	WCH X T	1.802***	NS	0.040***	0.013***

The data provided above represent the Mean ± SE ($n = 5$)

WW Well-watered, *DS* Drought stress, *RWF* Weed free rice, *RA* Rice + *A. paronychioides*, *RAH* Rice + *A. paronychioides* + Herbicide, *RE* Rice + *E. colona*, *REH* Rice + *E. colona* + Herbicide, *RWC* relative water content, *MSI* membrane stability index

Distinct lower letters in a column designates a significant difference at $P = 0.05$. *, **, and *** designates significant difference at $P < 0.05$, 0.01, and 0.001 levels, respectively, while "ns" indicates non-significance. WCH: refers to combinations of weeds, crop, and herbicide; T: denotes treatment; and WCH X T: represents the interaction between combinations of weeds, crop, herbicide, and treatment

herbicide-unsprayed controls due to the reduction of weed stress caused by herbicide application (Table 2).

Chlorophyll a and b

There was a noteworthy variation seen for chlorophyll-a and chlorophyll-b among WCH ($P < 0.001$), T ($P < 0.001$), and WCH X T ($P < 0.05$). The chlorophyll a level in weed-free rice were 4.24 mg g^{-1} FW and 3.16 mg g^{-1} FW in WW and DS, respectively. The chlorophyll b level in weed-free rice were 2.03 mg g^{-1} FW and 1.51 mg g^{-1} FW in WW and DS, respectively (Table 2). *A. paronychioides* interference resulted in a reduction of 39.89% and 59.80% in chl-a content under WW and DS conditions, respectively, while chl-b content decreased by 51.81% and 68.91% under WW and DS, respectively. *E. colona* interference resulted in a reduction of 52.56% and 68.92% in chl-a content under WW and DS conditions, respectively, while chl-b content decreased by 58.22% and 72.70% under WW and DS, respectively. Nevertheless, in comparison to their respective controls where herbicide was not applied, rice grown with *A. paronychioides* (chl-a: 2.62% and 28.32%; chl-b: 14.68% and 39.15% in WW and DS conditions, respectively) and *E. colona* (chl-a: 31.84% and 5.32%; chl-b: 35.43% and 6.63% in WW and DS conditions, respectively) exhibited a significant rise in chl-a and b content attributed to the alleviation of weed stress resulting from herbicide application (Table 2).

The study showed that *E. colona* caused greater reductions in RWC, MSI, and chlorophyll a & b in rice compared to *A. paronychioides*, with DS exacerbating these effects more than WW conditions. This suggests that rice is highly vulnerable to *E. colona* under DS, likely due to reduced herbicide efficacy.

Gas exchange parameters

The rate of photosynthetic (P*N*) showed significant differences for WCH, T, and WCH X T ($P < 0.001$). Under WW and DS conditions, the P*N* values of weed-free rice were recorded as 22.70 and 14.38 (μmol CO_2 $m^{-2}s^{-1}$), respectively. *A. paronychioides* interference led to significant reductions of 48.12% and 58.46% in P*N* in WW and DS, respectively. *E. colona* interference diminished PN to 59.49% and 63.41% in WW and DS, respectively, compared to

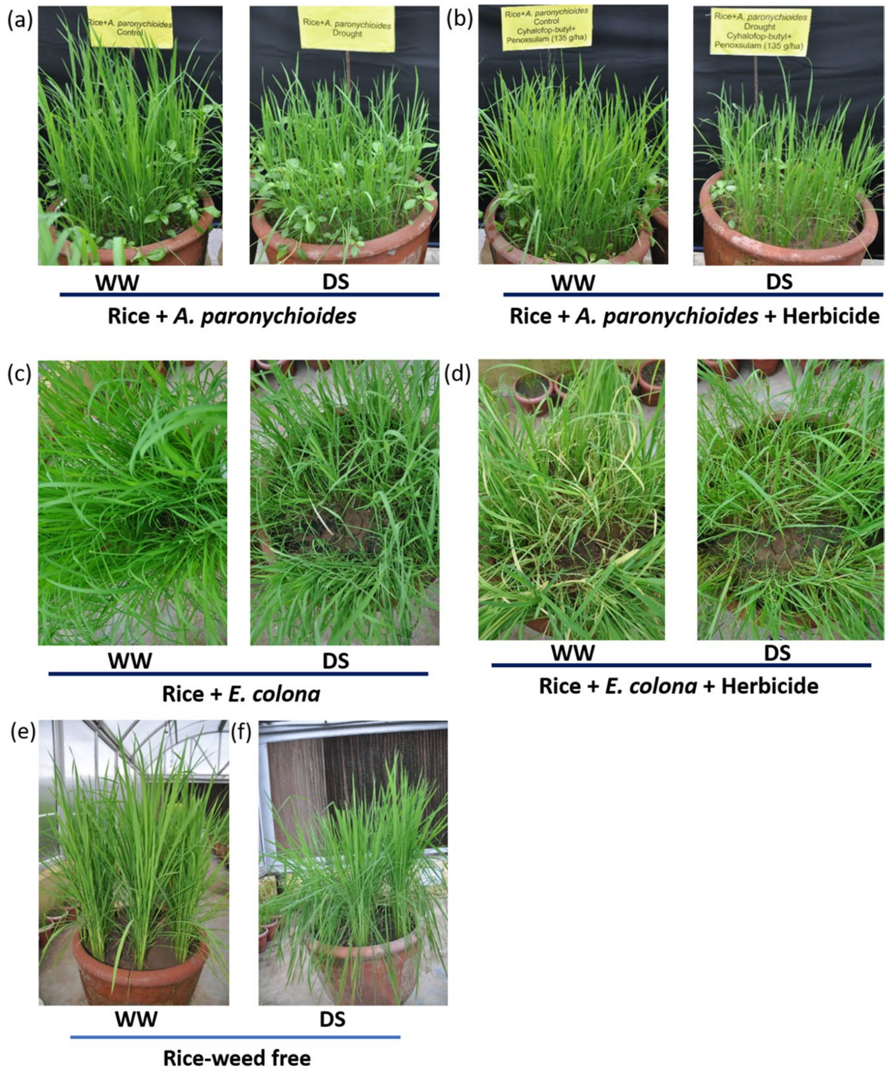

Fig. 1 Effect of drought and weed control treatments on rice morphological and physiological responses. Rice with *A. paronychioides* (**a** and **b**), rice with *E. colona* (**c** and **d**) and weed free rice (**e** and **f**) under well-watered (WW) and drought stress (DS)

the weed-free control. Compared to their respective controls without herbicide application, rice plants grown with *A. paronychioides* (38.81% and 35.03% in WW and DS conditions, respectively) and *E. colona* (13.59% and 13.24% in WW and DS, respectively) exhibited notable enhancements in their P*N* due to reduced weed stress induced by herbicide application.

Significant variations ($P<0.001$) in stomatal conductance (g*s*) were observed among WCH, T, and WCH × T. The g*s* values in WW and DS conditions in weed-free rice were 0.64 and 0.33 [mol (H_2O) $m^{-2}s^{-1}$], respectively (Fig. 2b). Furthermore, the presence of *E. colona* resulted in the highest reduction (56.77%) in g*s* under DS compared to the weed-free control. Herbicide application led to a reduction of weed stress on rice which caused a significant enhancement in the g*s* of rice grown with *A. paronychioides* (5.88% and 27.71% at WW and DS, respectively) and *E. colona* (11.76% and 30.77% at WW and DS, respectively) relative to their respective herbicide unsprayed controls.

A noteworthy difference was observed for *E* for WCH ($P<0.001$), T ($P<0.001$), and WCH X T ($P<0.05$). Under WW and DS, the *E* values for weed-free rice were 5.43 and 9.49 [mol (H_2O) $m^{-2}s^{-1}$], respectively (Fig. 2c). *A. paronychioides* led to reductions of 32.21% and 45.31% in *E* under WW and DS, while *E. colona* caused decreases to 48.96% and 59.64% in WW and DS, respectively, compared to the weed-free control. The use of

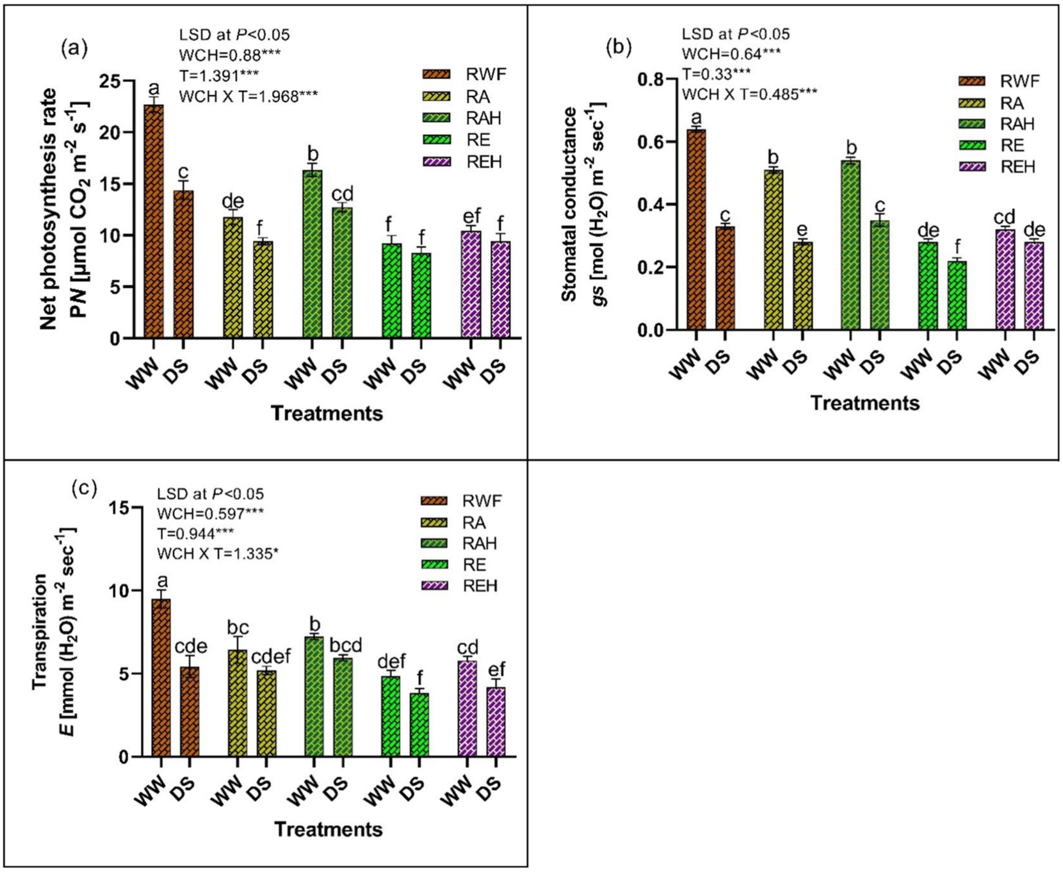

Fig. 2 Effect of drought stress and weed control treatments on rate of photosynthesis (**a**), stomatal conductance (**b**) and transpiration (**c**) of rice in well-watered (WW) and drought stress (DS) conditions. RWF: weed free rice; RA: rice + *A. paronychioides*; RAH: rice + *A. paronychioides* + herbicide; RE: rice + *E. colona*; REH: rice + *E.* colona + herbicide. The data provided above represent the Mean ± SE ($n = 5$). Distinct lower letters in a column designates a significant difference at $P = 0.05$. *, **, and *** specifies significant difference at $P < 0.05$, 0.01, and 0.001 levels, respectively, while "ns" specifies non-significance. WCH: refers to combinations of weeds, crop, and herbicide; T: denotes treatment; and WCH X T: represents the interaction between combinations of weeds, crop, herbicide, and treatment

herbicides led to a decrease in weed stress on rice, which in turn led to a notable improvement in the *E* of rice grown with *A. paronychioides* (12.54% and 14.84% at WW and DS, respectively) and *E. colona* (19.68% and 9.05% at WW and DS, respectively) when compared to herbicide-unsprayed controls.

The study found that rice photosynthesis declined more severely with *E. colona* than with *A. paronychioides*, and this decline was further exacerbated by DS compared to WW conditions. This indicates that rice is particularly vulnerable to *E. colona* under DS, likely due to decreased herbicide efficacy.

Oxidative stress markers

Superoxide ion

Superoxide ion exhibited a significant variance across WCH ($P < 0.001$), T ($P < 0.001$) and WCH X T ($P < 0.01$) was noted. In WW and DS, the superoxide ion levels in weed-free rice were 3.50 and 6.06 µmol g^{-1} FW, respectively (Fig. 3a). Superoxide ion levels under WW and DS, respectively, increased significantly by 1.15 and 1.63 times in the existence of *A. paronychioides* than weed-free

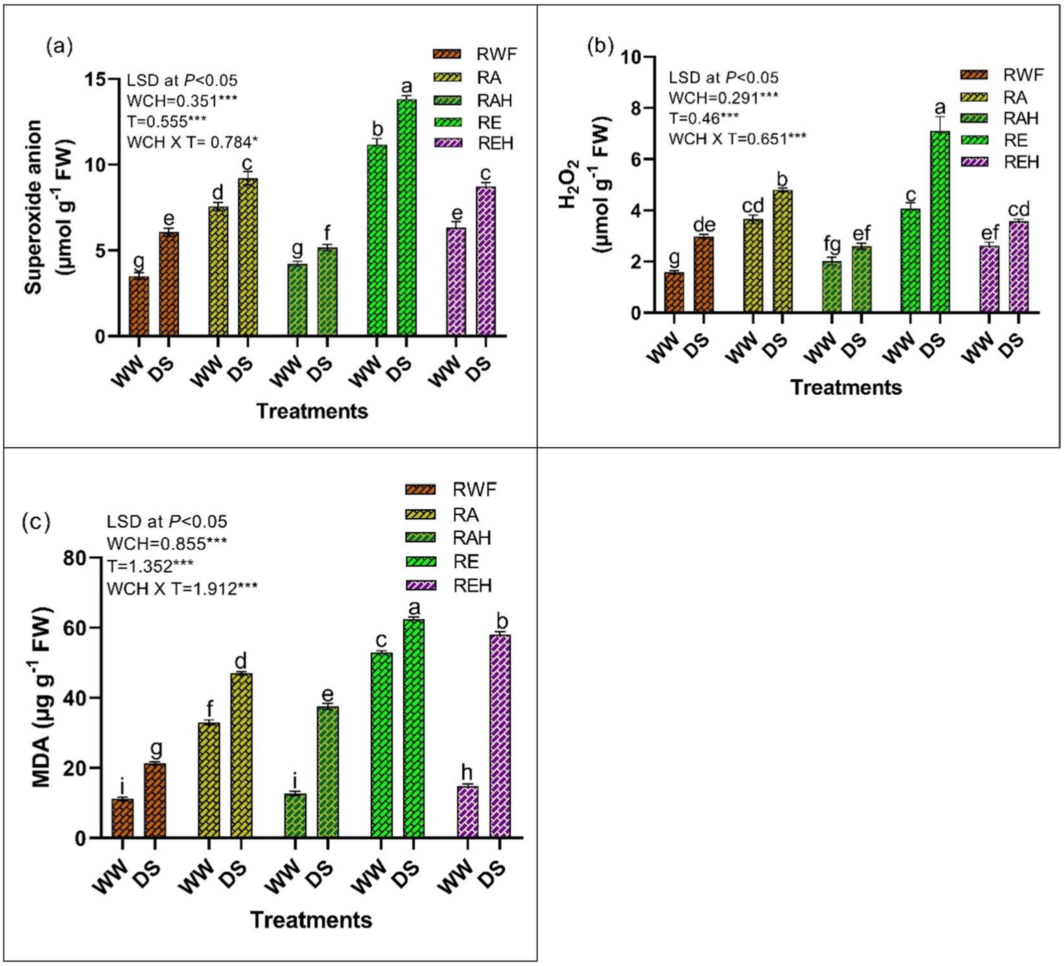

Fig. 3 Effect of drought stress and weed control treatments on superoxide ion content (**a**), H_2O_2 content (**b**) and MDA content (**c**) of rice in well-watered (WW) and drought stress (DS) conditions. RWF: weed free rice; RA: rice + *A. paronychioides*; RAH: rice + *A. paronychioides* + herbicide; RE: rice + *E. colona*; REH: rice + *E.* colona + herbicide. The data provided above represent the Mean ± SE ($n = 5$). Distinct lower letters in a column indicates a significant difference at $P = 0.05$. *, **, and *** indicates significant difference at $P < 0.05$, 0.01, and 0.001 levels, respectively, while "ns" specifies non-significance. WCH: refers to combinations of weeds, crop, and herbicide; T: denotes treatment; and WCH X T: represents the interaction between combinations of weeds, crop, herbicide, and treatment

control. Similarly, compared to weed-free control, *E. colona* interference elevated the superoxide ion level to 2.19 and 2.95 times in WW and DS, respectively. In comparison to their respective herbicide unsprayed controls, the superoxide ion content in rice planted with *A. paronychioides* (44.12% and 43.98% at WW and DS, respectively) and *E. colona* (43.28% and 36.95% at WW and DS, respectively) was significantly reduced upon herbicide application.

H_2O_2

A significant variation WCH, T and WCH X T ($P < 0.001$) was noted for H_2O_2 content. The H_2O_2 content values under WW and DS conditions in weed-free rice were 1.59 and 2.98 (µmol g^{-1} FW), respectively. A significant rise of 1.30fold and 2.02fold in H_2O_2 content was observed in the presence of *A. paronychioides* in WW and DS, respectively, compared to the weed-free control (Fig. 3b). Likewise, *E. colona* interference led to an increase in H_2O_2 level to 1.56fold and 3.47fold in WW and DS, respectively, than weed-free control. The application of herbicide reduced weed stress on rice, resulting in a significant decrease in H_2O_2 levels in rice grown with *A. paronychioides* (by 45.05% and 45.94% in WW and DS conditions, respectively) and *E. colona* (by 35.85% and 49.72% in WW and DS, respectively), compared to their respective controls without herbicide application.

Lipid peroxidation (MDA content)

A significant effect for MDA content across WCH, T and WCH X T ($P < 0.001$) was noticed. In rice without weeds, the MDA content values were 11.17 µg g^{-1} FW in WW and 21.45 µg g^{-1} FW under DS conditions. In both WW and DS conditions, the presence of *A. paronychioides* resulted in a remarkable upsurge of 1.95fold and 3.21fold in MDA content, respectively, compared to the weed-free control (Fig. 3c). Similarly, *E. colona* interference elevated the MDA content to 3.73fold and 4.59fold in WW and DS, respectively, than weed-free control. The application of herbicide reduced weed stress on rice, resulting in a significant decrease in MDA content in rice grown with *A. paronychioides* (61.27% and 20.28% in WW and DS conditions, respectively) and *E. colona* (72.06% and 6.94% in WW and DS, respectively), compared to their respective controls without herbicide application.

The study found that *E. colona* caused production of higher levels of stress markers (Superoxide ion, H_2O_2, MDA) in rice compared to *A. paronychioides*, with DS exacerbating these effects more than WW conditions. This suggests that rice is highly vulnerable to *E. colona* under DS, likely due to reduced herbicide efficacy.

Antioxidant enzymes

Significant differences in SOD, CAT, and POD were observed among WCH ($P < 0.001$) and T ($P < 0.001$). However, while the interaction effects (WCH × T) were significant for SOD and CAT ($P < 0.001$), and non-significant for POD.

In rice without weeds, there was a significant 65.94% increase in SOD in DS than WW weed-free control. In the existence of *A. paronychioides*, SOD levels increased by 2.45fold and 3.11fold in WW and DS conditions, respectively, compared to the weed-free control (Fig. 4a). However, *E. colona* interference remarkably raised SOD levels to 1.26fold and 2.60fold in WW and DS, respectively, than control. Herbicide application alleviated weed stress on rice, leading to a considerable reduction in SOD levels in rice cultivated with *A. paronychioides* (63.21% and 15.86% during WW and DS conditions, respectively) and *E. colona* (21.64% and 21.16% during WW and DS, respectively), compared to their respective controls untreated with herbicide.

In rice without weeds, CAT showed a significant increase of 40.51% under DS than WW weed-free control. In the occurrence of *A. paronychioides*, CAT levels increased by 1.02-fold and 1.20-fold in WW and DS, respectively, compared to the weed-free control (Fig. 4b). Likewise, in WW and DS, respectively, *E. colona* occurrence greatly raised CAT to 68.12% and 78.71% in comparison to the control. Herbicide application reduced weed stress on rice, resulting in a significant decrease in CAT levels in rice grown with *A. paronychioides* (46.49% and 5.62% during WW and DS conditions, respectively) and *E. colona* (2.60% and 1.48% during WW and DS, respectively), compared to their respective controls without herbicide application.

POD in weed-free rice increased (41.54%) significantly under DS compared to WW. *A. paronychioides* interference remarkably accelerated POD by 1.80fold and 2.48fold in WW and DS, respectively, compared to weed-free control (Fig. 4c). In contrast, compared to weed-free control, *E. colona* existence upsurged the POD to 66.56% and 1.03fold in WW and DS, respectively. Herbicide application reduced weed stress on rice, resulting in a significant decrease in POD levels in rice grown with *A. paronychioides* (46.38% and 48.38% during WW and DS, respectively) and *E. colona* (10.77% and 26.52% during WW and DS, respectively), compared to their respective controls without herbicide application.

Non enzymatic antioxidants

Proline

Significant variability among WCH, T and WCH X T ($P < 0.001$) was noticed for proline content. The proline quantity values under WW and DS conditions in weed-free rice were 14.67 mg g^{-1}

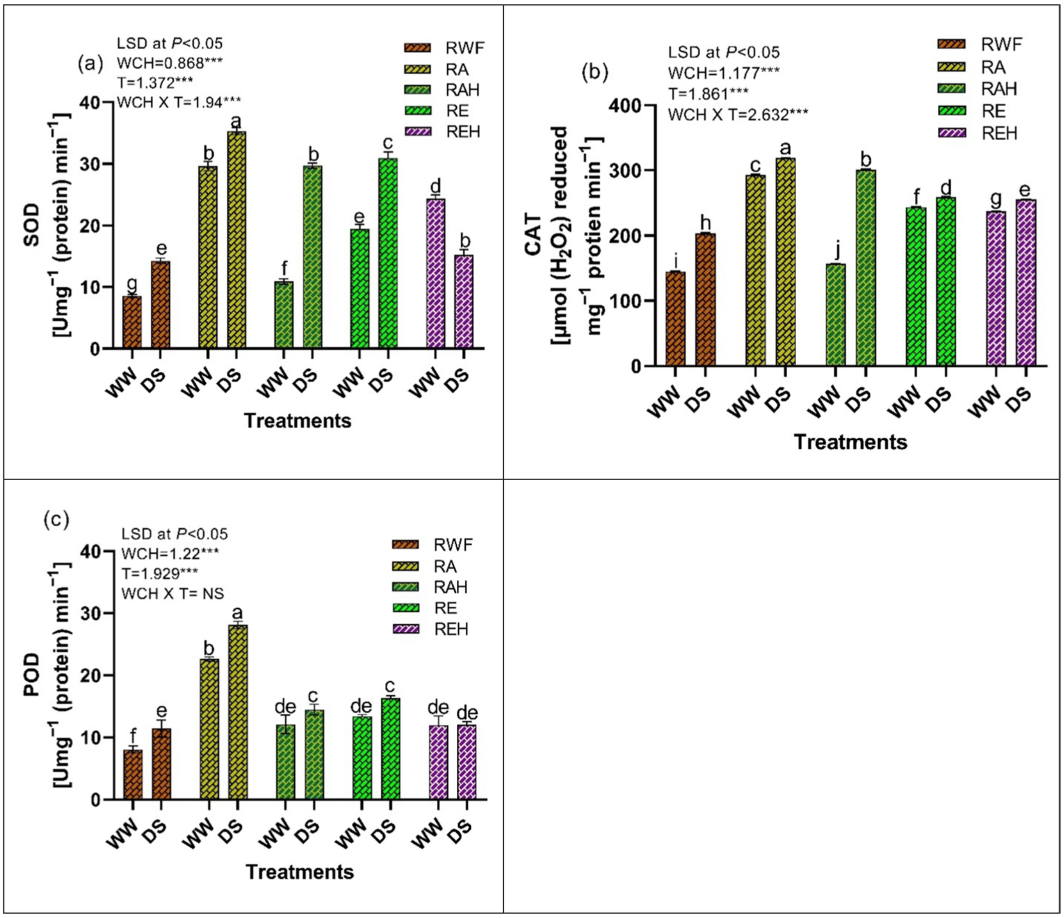

Fig. 4 Effect of drought stress and weed control treatments on superoxide dismutase (**a**), catalase (**b**) and peroxidase (**c**) of rice in well-watered (WW) and drought stress (DS) conditions. RWF: weed free rice; RA: rice + *A. paronychioides*; RAH: rice + *A. paronychioides* + herbicide; RE: rice + *E. colona*; REH: rice + *E.* colona + herbicide. The data provided above represent the Mean ± SE ($n = 5$). Distinct lower letters in a column specifies a significant difference at $P = 0.05$. *, **, and *** indicates significant difference at $P < 0.05$, 0.01, and 0.001 levels, respectively, while "ns" specifies non-significance. WCH: refers to combinations of weeds, crop, and herbicide; T: denotes treatment; and WCH X T: represents the interaction between combinations of weeds, crop, herbicide, and treatment

FW and 20.33 mg g^{-1} FW, respectively. Proline levels in WW and DS, respectively, significantly increased 1.18 and 3.00 times in the presence of *A. paronychioides* compared to weed-free control (Supplementary Fig. 3a). In contrast, compared to weed-free control, *E. colona* existence elevated the proline to 68.18% and 2.52fold in WW and DS, respectively. Herbicide application decreased weed stress on rice, resulting in a significant reduction in proline accumulation in rice grown with *A. paronychioides* (51.04% and 29.55% during WW and DS, respectively) and *E. colona* (35.14% and 41.94% during WW and DS, respectively), compared to their respective controls without herbicide application.

Total phenolic content (TPC)

A significant variation WCH, T and WCH X T ($P < 0.001$) was noted for TPC. In WW and DS, the TPC values for weed-free rice were 11.22 mg g^{-1} FW and 19.07 mg g^{-1} FW, respectively. In comparison to weed-free control, the existence of *A. paronychioides* resulted in a notable increase in TPC content in WW and DS of 1.78 and 2.51fold, respectively. (Supplementary Fig. 3b). On the other hand, compared to weed-free control, *E. colona* existence upsurged the TPC content to 1.03 and 1.61fold in WW and DS, respectively. The application of herbicide reduced weed stress on rice, resulting in a significant decrease in TPC in rice grown with *A. paronychioides* (58.72% and 62.63% during WW and DS conditions, respectively) and *E. colona* (42.72% and 42.68% during WW and DS, respectively).

Ascorbic acid content (asa)

Ascorbic acid varied significantly for WCH ($P<0.001$), T ($P<0.001$) and WCH X T ($P<0.05$). The AsA values in weed-free rice were 9.25 µg g^{-1} FW and 14.58 µg g^{-1} FW under WW and DS, respectively. The content of AsA significantly increased in both WW and DS in the existence of *A. paronychioides*, by 1.58 and 2.04fold, respectively, compared to the weed-free control (Supplementary Fig. 3c). In contrast to weed-free control, *E. colona* existence amplified the AsA level to 90% and 1.27fold in WW and DS, respectively. Herbicide application decreased weed stress on rice, resulting in a significant reduction in AsA levels in rice grown with *A. paronychioides* (54.70% and 57.10% during WW and DS conditions, respectively) and *E. colona* (25.59% and 28.17% during WW and DS, respectively), compared to their respective controls without herbicide application.

The study revealed that *E. colona* led to higher levels of non-enzymatic antioxidants (total phenolic content, ascorbic acid, proline) in rice compared to *A. paronychioides*, with DS amplifying these effects more than WW conditions.

Starch content

The starch content exhibited significant differences among WCH and T ($P<0.001$), however the interaction effect (WCH × T) was not significant. The starch levels in weed-free rice were 22.21 mg/g DW under DS and 26.23 mg/g DW in WW, respectively. *A. paronychioides* interference significantly reduced the starch content in WW and DS, respectively, by 36.98% and 44.87%, than weed-free control (Fig. 5a). However, in comparison to weed-free control, *E. colona* existence significantly decreased the starch to 53.89% and 69.35% in WW and DS, respectively. The application of herbicide reduced weed stress on rice, leading to a significant increase in rice starch levels

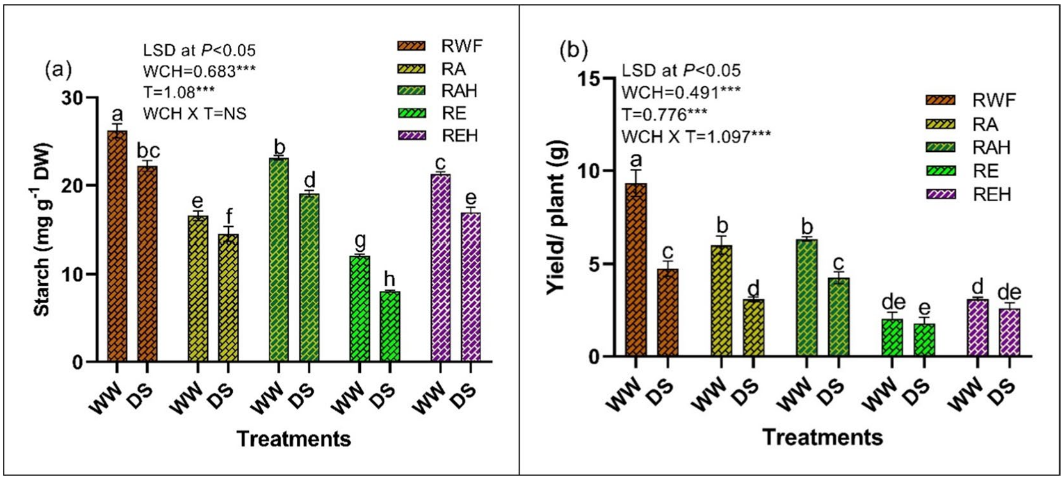

Fig. 5 Effect of drought stress and weed control treatments on starch (**a**) and yield (**b**) of rice in well-watered (WW) and drought stress (DS) conditions. RWF: weed free rice; RA: rice + *A. paronychioides*; RAH: rice + *A. paronychioides* + herbicide; RE: rice + *E. colona*; REH: rice + *E.* colona + herbicide. The data provided above represent the Mean ± SE ($n=5$). Distinct lower letters in a column indicates a significant difference at $P=0.05$. *, **, and *** indicates significant difference at $P<0.05$, 0.01, and 0.001 levels, respectively, while "ns" specifies non-significance. WCH: refers to combinations of weeds, crop, and herbicide; T: denotes treatment; and WCH X T: represents the interaction between combinations of weeds, crop, herbicide, and treatment

when grown with *A. paronychioides* (39.96% and 31.84% during WW and DS conditions, respectively) and *E. colona* (75.78% and 1.1-fold during WW and DS, respectively), compared to their respective controls without herbicide application.

Rice grain yield

There was a significant difference across WCH, T and WCH X T ($P<0.001$) noticed for yield. The yield values for weed-free rice in WW and DS conditions were 9.32 and 4.74 (g/plant), respectively. Whereas, weed interference caused a notable reduction in yield when exposed to DS. Compared to the weed-free control, the interference of *A. paronychioides* led to a drop in yield by 32.40% under WW and by 66.81% in DS (Fig. 5b). In contrast, compared to weed-free control, *E. colona* existence decreased the yield to 78.22% and 80.86% in WW and DS, respectively. The application of herbicide reduced weed stress on rice, leading to a significant increase in rice yield when grown with *A. paronychioides* (5.12% and 37.38% during WW and DS, respectively) and *E. colona* (52.05% and 45.18% during WW and DS, respectively), compared to their respective controls without herbicide application.

The study found that *E. colona* caused greater reductions in rice starch content and grain yield compared to *A. paronychioides*, with DS worsening these effects more than WW conditions. This suggests that rice is highly vulnerable to *E. colona* under DS, due to reduced herbicide efficacy.

Weeds growth and biomass traits

Significant variations were observed in the growth and biomass traits of weed species (*A. paronychioides* and *E. colona*) in reaction to herbicide application under DS. A significant ($P<0.001$) Weed and Herbicide (WH) (weeds: *A. paronychioides*; *E. colona* and herbicide) and WH X T effect was noticed for weed fresh weight. However, the T (control and drought) was non-significant. The *A. paronychioides* fresh weight values were 8.53 g and 6.47 g in WW and DS, respectively. Similarly, *E. colona* fresh weight values were 5.50 g and 4.93 g in WW and DS respectively. Herbicide application led to a decrease in the weed's fresh weight i.e., *A. paronychioides* (70.70% and 61.86% at WW and DS, respectively) and *E. colona* (63.03% and 41.89% at WW and DS, respectively) in comparison to their respective herbicide unsprayed controls (Table 3).

A notable variation was observed among WH ($P<0.001$), T ($P<0.05$), and the interaction between WH and T ($P<0.01$) for weed dry weight. Herbicide application led to a decline in the weeds

Table 3 Effect of weed control treatments on weed growth and biomass traits under drought stress

Weeds and herbicide combination (WH)	Treatment (T)	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Root length (cm)
A. paronychioides	WW	8.53 ± 0.98a	0.96 ± 0.13a	96.95 ± 1.44a	9.67 ± 0.33 cd
	DS	6.47 ± 0.28b	0.65 ± 0.05b	63.67 ± 1.68b	11.67 ± 0.88bc
A. paronychioides + Herbicide	WW	2.50 ± 0.17d	0.40 ± 0.03 cd	43.63 ± 1.13d	7.33 ± 0.67e
	DS	2.47 ± 0.23c	0.31 ± 0.02bc	32.17 ± 1.80de	9.00 ± 0.58de
E. colona	WW	5.50 ± 0.23bc	0.63 ± 0.06b	54.43 ± 1.44c	13.00 ± 0.58ab
	DS	4.93 ± 0.48d	0.57 ± 0.12d	40.77 ± 0.94f	14.33 ± 0.88a
E. colona + Herbicide	WW	2.03 ± 0.18d	0.40 ± 0.02 cd	37.50 ± 0.92e	11.67 ± 0.88bc
	DS	2.87 ± 0.35d	0.39 ± 0.03 cd	32.77 ± 1.34f	13.33 ± 0.33ab
LSD = $P<0.05$	WH	***	***	***	***
	T	NS	*	***	**
	WH X T	***	**	***	NS

The data provided above represent the Mean ± SE ($n=5$)

Distinct lower letters in a column designates a significant difference at $P=0.05$. *, **, and *** designates significant difference at $P<0.05$, 0.01, and 0.001 levels, respectively, while "ns" designates non-significance

WW Well-watered, *DS* drought stress, *WCH* refers to combinations of weeds, crop, and herbicide, *T* denotes treatment, *WCH X T* represents the interaction between combinations of weeds, crop, herbicide, and treatment dry weight i.e., *A. paronychioides* (58.13% and 51.55% at WW and DS, respectively) and *E. colona* (36.84% and 31.76% at WW and DS, respectively) in comparison to their respective herbicide unsprayed controls (Table 3).

A significant variation for WH, T and WH X T ($P < 0.001$) was noticed for the leaf area. Herbicide application led to a reduction in the weeds leaf area i.e., *A. paronychioides* (55.00% and 49.48% at WW and DS, respectively) and *E. colona* (31.11% and 19.62% at WW and DS, respectively) compared to their respective herbicide unsprayed controls (Table 3).

Root length varied significantly for WH ($P < 0.001$) and T ($P < 0.01$) was noted for root length. Whereas, the interaction effect (WH X T) was non-significant. Herbicide application led to a decrease in the weeds root length i.e., *A. paronychioides* (24.14% and 22.86% at WW and DS, respectively) and *E. colona* (10.26% and 6.98% at WW and DS, respectively) compared to their respective herbicide unsprayed controls (Table 3).

Remarkably, the growth and biomass parameters of *A. paronychioides* and *E. colona* were shown to decrease upon the application of herbicide under DS. Whereas, the intensity of drop was significantly lesser in *E. colona* than in *A. paronychioides*, signifying a higher tolerance against DS. The level of minimal growth decline of *E. colona* in DS is thought to have a direct correlation with the lowered herbicide efficacy. This observation led us to infer that *E. colona* exhibits more effective and pronounced adaptive mechanisms to DS than *A. paronychioides*. Additionally, the application of herbicide was less effective in drought conditions.

Principal component analysis (PCA)

The first two principal components (PCs) obtained from the PCA of the rice's responses to several treatments had eigenvalues more than 1 and were statistically significant (Supplementary Table 1; Fig. 6). The PC-1 and 2 together explained 88.2% (PC-1:72.1% and PC-2: 16.1%) of variation (Fig. 6) among 12 parameters. In PC-1, the highest variance

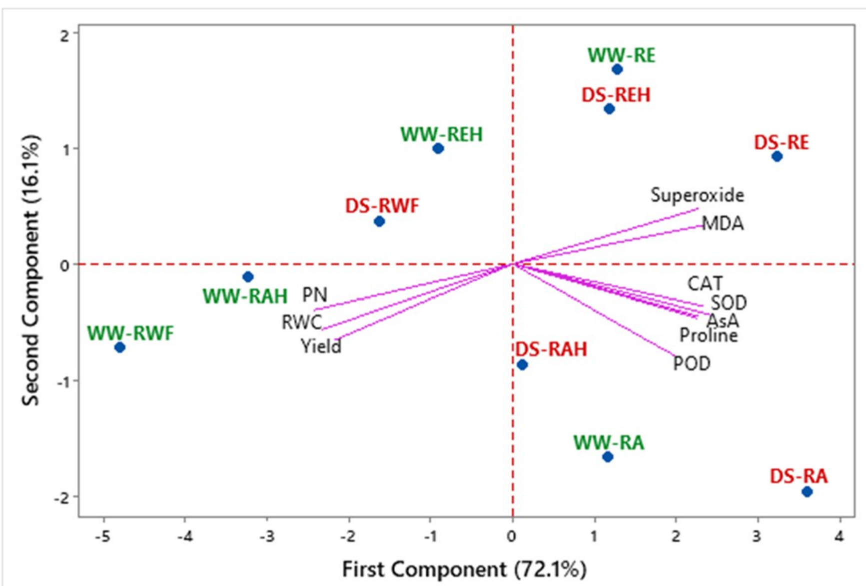

Fig. 6 The principal component analysis (PCA) of physiological, biochemical traits and yield of rice in various weed control treatments under WW and DS conditions. RWF: weed free rice; RA: rice + *A. paronychioides*; RAH: rice + *A. paronychioides* + herbicide; RE: rice + *E. colona*; REH rice + *E.* colona + herbicide; WW: well-watered condition; DS: drought stress; RWC: relative content; MDA: malondialdehyde content, Superoxide: superoxide ion content; Proline: proline content; AsA: ascorbic acid content; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; PN: rate of photosynthesis; Yield: grain yield

was attributed to SOD (0.337), CAT (0.323), MDA (0.322), and Proline (0.316), while total PN (-0.336), RWC (-0.322), and yield (-0.300) contributed the least variance. Conversely, in PC-2, superoxide (0.296) and MDA (0.208) contributed the most variance, whereas POD (-0.488) and yield (-0.404) contributed to the low variance.

Pearson's correlation analysis

The results of this study were additionally confirmed through Pearson's correlation analysis. This analysis was examined for 15 physiological and biochemical traits of rice under different treatment conditions, encompassing both WW and DS conditions. The correlation matrices are presented in Fig. 7. The analysis indicated significant positive correlations ($P<0.01$) between yield and POD (0.946**), RWC (0.903**), total chlorophyll (0.857**), and starch content (0.786**), while significant negative correlations ($P<0.01$ and $P<0.05$) were observed with superoxide (-0.794**), MDA (-0.760*), and H_2O_2 (-0.699*). Conversely, PN showed significant positive correlations ($P<0.01$) with RWC (0.932**), total chlorophyll (0.904**), starch content (0.831**), and MSI (0.828**), while significant negative correlations ($P<0.01$ and $P<0.05$) were found with superoxide (-0.798**), MDA (-0.768**), CAT (-0.760*), SOD (-0.701*), and H_2O_2 (-0.727*).

Discussion

Drought stress stands as a formidable abiotic challenge globally, impacts crop productivity (Lesk et al. 2016; Matiu et al. 2017). It has been documented that water is the key environmental component that influences the yield and quality of plants (Saudy et al. 2020; El-Metwally and Saudy 2021a). In this context, supplying plants with the adequate quantity of water improved and sustained metabolic activities, productivity and water use efficiency (Saudy et al. 2021a,

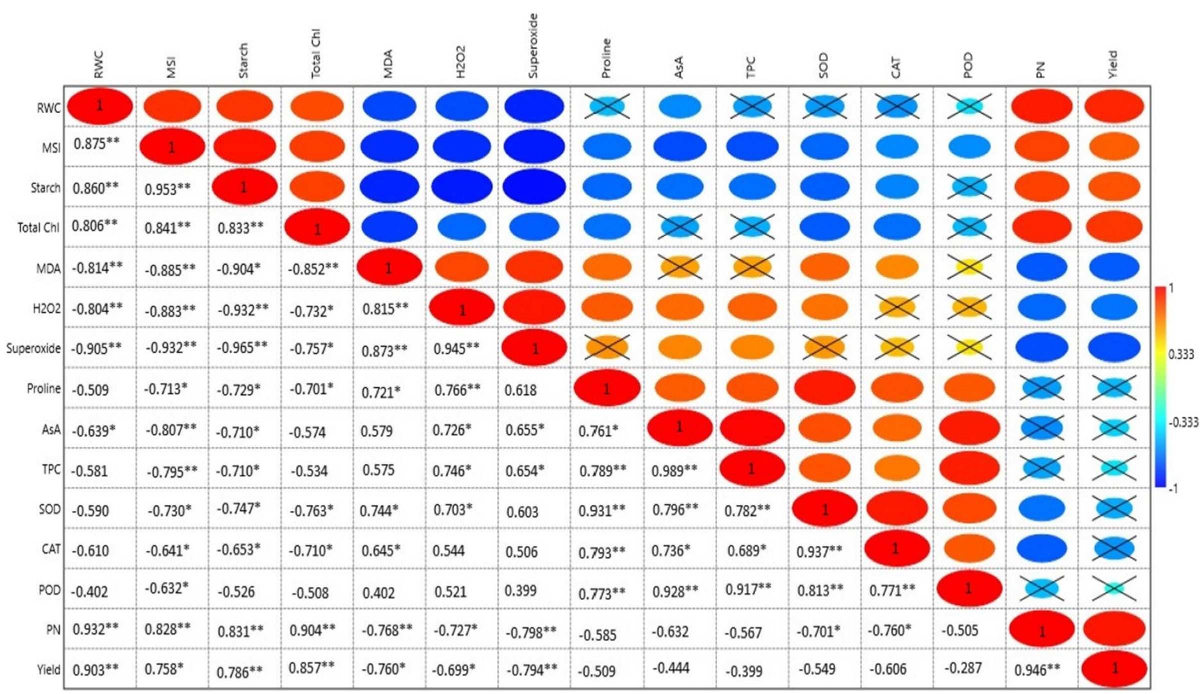

Fig. 7 Correlogram showing the relationship between various physiological, biochemical traits and yield of rice affected by weed control treatments in WW and DS conditions. Asterisks (*, **, ***) indicate significance at <0.05; <0.01; <0.001, respectively. RWC: relative water content; MSI: membrane stability index; Total chl: total chlorophyll content; Proline: proline content; AsA: ascorbic acid content; TPC: total phenolic content; MDA: malondialdehyde content; H_2O_2: H_2O_2 content, Superoxide: superoxide ion content; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; Starch: starch content; PN:rate of photosynthesis; Yield: grain yield

2023a; Ramadan et al. 2023a; Ali et al. 2024). In both irrigated and non-irrigated agricultural contexts, weeds persist as a pivotal biological limitation despite advancements in crop varieties (Mahajan et al. 2015). The drought stress, significantly impairs the efficacy of herbicides in managing weeds, often resulting in reduced effectiveness (Weller et al. 2019; Saudy and El-Metwally 2023). In the current examination, we investigate how drought stress affects the performance of the herbicide (cyhalofop + penoxsulam) against *A. paronychioides* and *E. colona*. Additionally, we assess the potential consequences of inadequate weed control, including the survival of weeds and their adverse effects on rice's physiological, biochemical traits, and yield.

In the context of weed management, drought-affected weeds create difficulties for their management using post-emergent herbicides. Herbicides require soil moisture to function effectively, but their efficacy can be diminished by drought (Singh et al. 2015). Herbicides struggle to penetrate plant tissues due to increased cuticle thickness and leaf hairs caused by drought-induced responses (Patterson 1995). These traits influence the growth of crops and weeds and the recovery process after herbicide application (Saudy and Mubarak 2015). In drought-prone areas, herbicide volatilization intensifies, while regular rainfall limits safe herbicide application windows in agriculture (Froud-Williams 1996). These interconnected factors create complex challenges in weed management. Likewise, in the current investigation, the efficacy of the herbicide (cyhalofop + penoxsulam) was reduced against, *A. paronychioides* and *E. colona*, during drought conditions. Consequently, the weeds that managed to survive due to this diminished herbicide efficacy exerted a noteworthy adverse impact on the physiological, biochemical, and yield-related characteristics of rice.

To retain normal functionality, plants that are growing in challenging environments modify their metabolic processes (Hussain et al. 2019; Ansari et al. 2021; Saudy et al. 2021b). Drought adversely affects plant morphology, physiology, and yield by reducing leaf area, chlorophyll content, and RWC while increasing ROS (Lata and Prasad 2011; Salehi-Lisar and Bakhshayeshan-Agdam 2016; El-Bially et al. 2018; Doklega et al. 2024). Plants go through several morpho-physiological and biochemical changes to lessen these negative effects of drought (Mishra et al. 2018; Saudy et al. 2023b; El-Metwally et al. 2021; Ramadan et al. 2023b). In this study, it was observed that the reduction in various rice traits was more pronounced in the occurrence of *E. colona* compared to *A. paronychioides*. This effect was consistent across both herbicide-treated and non-treated rice, with DS conditions exacerbating the decrease in traits more significantly than under WW conditions. This specifies that rice is extremely susceptible to *E. colona* under DS. The degree of growth lessening under DS is believed to be directly linked to the diminished efficacy of herbicides against *E. colona*.

RWC emerges as a pivotal physiological marker influencing water potential, stomatal resistance, and transpiration (Hartmann et al. 2013; Saudy and El-Metwally 2019; Makhlouf et al. 2022). Weed presence in soybean led to reduced leaf RWC, which improved upon herbicide application (Khaffagy et al. 2022). Notably, decreased RWC and MSI (membrane stability index) are associated with drought stress (Bangar et al. 2019).

Our study emphasizes that rice, when subjected to DS, exhibits a greater decline in RWC and MSI in the occurrence of *E. colona* than *A. paronychioides*, regardless of herbicide application.

Water scarcity has a detrimental negative effect on various physiological characteristics of rice, i.e., the gas exchange parameters (Zhu et al. 2020). Additionally, weed-infested soybean plants had a lower rate of assimilation of carbon dioxide, which led to a notable drop in carbon allocation (Saudy and El-Metwally 2009; McKenzie-Gopsill et al. 2019). Correspondingly, our study demonstrates distinctive decreases in these (P_N, gs, and E) rice traits due to *E. colona* in contrast to *A. paronychioides*, regardless of herbicide treatment, under DS conditions than WW conditions. Photosynthesis is notably one of the primary processes affected by drought stress, as supported by the findings of Lawlor and Cornic (2002). Drought-induced stomatal closure limits CO_2 uptake, directly reducing photosynthetic activity. However, beyond stomatal factors, drought also triggers alterations in the photosynthetic metabolism, disrupting the efficiency of photosystems, carbon assimilation, and energy production.

The low chlorophyll content is commonly considered a typical indication of oxidative stress triggered by drought conditions (Faisal et al. 2019). The deterioration in chlorophyll content caused by drought stress can be linked to the photooxidation of pigments and the deterioration of chlorophyll (Nezhadahmadi et al. 2013; El-Metwally et al. 2022a; Shaaban et al. 2023). Similarly, it was noticed that the level of reduction in chlorophyll-a and chlorophyll-b content of rice was more in the existence of *E. colona* than *A. paronychioides*, in both herbicide-treated and non-treated rice under DS than WW condition. Due to various stresses, the drop in photosynthetic pigment concentrations, such as chlorophyll, could directly limit the photosynthetic activities (Farooq et al. 2009; Saudy 2014; Abd–Elrahman et al. 2022; Hadid et al. 2023).

MDA, is a byproduct of lipid peroxidation, is a vital indicator of weed stress. Increased electrolyte leakage via the plasma membrane during weed infection can trigger the production of ROS (Tanaka et al. 1993). Plants may experience a number of negative effects from drought stress, including disruption of redox equilibrium and potential oxidative damage (El-Bially et al. 2022; Salem et al. 2022). Over production of ROS such as $O_2.^-$, OH and H_2O_2 results in oxidative stress. This can then result in membrane degradation, electron leakage, and lipid peroxidation. Furthermore, apart from damaging proteins and nucleic acids, ROS also prevents stomata closing and interferes with enzyme and photosynthesis (Polle 2001; Asada 2006; Jaspers and Kangasjärvi 2010; Maksup et al. 2014). Likewise, in our present study, the extent of enhancement of ROS (O_2^-, H_2O_2) and MDA generation was greater in rice due to *E. colona* than *A. paronychioides*, in both herbicide-treated and non-treated rice under DS than WW conditions.

To lower the detrimental effects of drought, plants trigger their defense system by enhancing the level of antioxidants (e.g., SOD, CAT, and POD) and non-enzymatic antioxidants (TPC and AsA) (Hussain et al. 2018). Antioxidant, AsA is essential in mitigating the harm induced by oxidative stress (Foyer and Noctor 2005). In numerous plant species, TPC levels (Wrobel et al. 2005) and proline (Mohammadi et al. 2018) may rise under abiotic stress. Several physiological alterations were noted under weed infestation. Among these, the excess generation of ROS and modifications in antioxidant substances are paramount. Weed infestation resulted in reduced activity of SOD, while levels of H_2O_2 and oxidized AsA were increased. It was observed that the lipid peroxidation, superoxide, and CAT activity remained unchanged in soybean, indicating no significant alteration (McKenzie-Gopsill et al. 2019), as were changes in leaf antioxidants such as AsA and glutathione and other antioxidant enzymes (Yamazaki 2010; Afifi and Swanton 2012; Gal et al. 2015; Agostinetto et al. 2017). The degree of APX, CAT and SOD were dropped with *Cyperus rotundus* L. competition in soybean (Darmanti et al. 2016). It creates a notable shift in proline accumulation (Jabran et al. 2018). Similar trends are evident in our study, wherein the occurrence of *E. colona* induced greater reductions in antioxidant enzyme accumulation, TPC, AsA, and proline content in rice compared to *A. paronychioides*, particularly under DS conditions with and without herbicide treatment.

The starch is broken down under DS to supply energy and carbon when photosynthetic activity is limited, resulting in lower starch content (Siaut et al. 2011). In our study, we found a greater decrease in rice starch content, particularly notable in the presence of *E. colona*. Both herbicide-treated and non-treated rice experienced a substantial reduction in starch content under DS compared to WW conditions, with *E. colona* causing more significant reductions than *A. paronychioides*. The study highlights various physiological changes in response to drought stress, including alterations in chlorophyll content, protein levels, starch, MDA, and proline, as well as variations in RWC, MSI, transpiration rate, and photosynthesis. These findings are consistent with previous research by Fukai and Cooper (1995) and Kumar et al. (2014), which documented similar physiological responses under drought conditions.

Rice production is significantly constrained by drought (Sasi et al. 2021). Likewise, it was noticed that under DS, rice exhibited a more significant yield decrease in *E. colona* than *A. paronychioides*. This phenomenon was observed in both herbicide-treated and untreated rice under DS, indicating rice's higher vulnerability to the negative impacts of *E. colona*. The degree of growth decline under stress is believed to directly correlate with the diminished effectiveness of herbicides against *E. colona*. The findings of this study align with previous research by Rollins et al. (2013), which demonstrated that plant physiological and yield traits are more severely impacted under combined stress conditions than when individual stresses are applied. This suggests that the interaction of multiple stress factors may exacerbate the negative effects on plant growth and productivity. The compounded stress likely overwhelms the plant's adaptive mechanisms, leading to a more significant reduction in physiological function and yield. This highlights the importance of considering combined stress scenarios in future research and crop management strategies, particularly in the context of climate change, where plants are likely to encounter multiple stressors simultaneously.

Unforeseen drought events disrupt water availability and the equilibrium of crop-weed competition, intensifying competition magnitude (Ramesh et al. 2017). Under DS, *Abutilon theophrasti* Medic. and *Anoda cristata* (L.) Schltdl. became more competitive with cotton (Patterson and Highsmith 1989). The adoption of direct-seeded rice, driven by water scarcity, is fostering the spread of troublesome grassy weeds such as *Dactyloctenium aegyptium*, *Eleusine indica*, *Leptochloa chinensis*, and weedy rice (*Oryza sativa*) in rice fields (Chauhan et al. 2014; Matloob et al. 2015). Similarly, our study found that *E. colona* has a greater impact on rice under drought stress (DS) conditions compared to *A. paronychioides*. This could be attributed to the C_4 photosynthetic pathway of *E. colona*. DS is a main abiotic stress that has a considerable impact to herbicides, primarily decreasing their efficacy (Weller et al. 2019). It has been documented that the effectiveness of herbicides decreases under DS (Miller and Norsworthy 2018; Skelton et al. 2016; Wu et al. 2019). Plants can survive the application of herbicides by naturally possessing the ability to metabolize (break down or detoxify) sufficient amounts of the herbicide or by augmenting this ability under conditions of environmental stress (Nandula et al. 2019; El-Metwally et al. 2022b). The study reveals that the efficacy of benzoylprop-ethyl herbicide was notably reduced under drought conditions, consistent with findings by Alizade et al. (2020). However, the current investigation indicates that the herbicide's effectiveness was diminished against both *A. paronychioides* and *E. colona*, with a more significant reduction observed for *E. colona*. This suggests that *E. colona* may possess a higher level of drought tolerance compared to *A. paronychioides*. The greater decrease in herbicide efficacy against *E. colona* highlights its potential resilience under water-limited conditions, which could impact its management in drought-affected areas. This emphasizes the need for targeted strategies to address the varying drought responses of different weed species to optimize herbicide use and improve weed control under challenging environmental conditions. Hence, it is imperative to comprehend how global climate change factors, such as drought, affect weeds and herbicide efficacy to optimize herbicide usage and achieve efficient weed management.

Conclusion

This study investigated the impact of drought stress on the efficacy of the herbicide cyhalofop + penoxsulam against the weeds *Alternanthera paronychioides* and *Echinochloa colona*. The results revealed that the herbicide's effectiveness was significantly reduced under drought conditions, with a more pronounced decline observed in controlling *Echinochloa colona* compared to *Alternanthera paronychioides*. This reduction in herbicide efficacy led to increased weed survival, particularly of *Echinochloa colona*, which in turn caused greater oxidative stress in rice plants, evidenced by decreased antioxidant enzyme activities and lower levels of non-enzymatic antioxidants. Additionally, rice starch content was more severely reduced in the presence of *Echinochloa colona* than *Alternanthera paronychioides*. The presence of *Echinochloa colona* exacerbated yield losses more significantly under drought stress, highlighting its competitive advantage under challenging conditions. To counter the intricate challenges posed by drought and weed infestations, a holistic understanding of their synergistic effects on crops is imperative. The findings underscore the need for integrated weed management strategies that account for the combined effects of drought and weed infestations. To improve weed control and mitigate yield losses, future research should focus on developing adaptive strategies and alternative management practices that can effectively address these synergistic challenges.

Acknowledgements The authors express gratitude to the ICAR-Directorate of Weed Research for the essential facilities and financial assistance.

Author contributions Dasari Sreekanth, Pawar Deepak Vishwanath, Shobha Sondhia, JS Mishra: Conceptualization Supervision, Project administration; Dasari Sreekanth, Pawar Deepak Vishwanath: Methodology; Dasari Sreekanth, Pawar Deepak Vishwanath, Survi Mahesh, Shobha Sondhia, C.R.

Chethan, P.S. Basavaraju: Validation; Dasari Sreekanth, Nagaraju Mukkamula, B Kiran Kumar: Formal analysis; Dasari Sreekanth: Investigation; Dasari Sreekanth, Pawar Deepak Vishwanath: Data Curation; Dasari Sreekanth, Survi Mahesh: Writing - Original Draft; Dasari Sreekanth, Pawar Deepak Vishwanath, Survi Mahesh, JS Mishra, PK Singh, Nagaraju Mukkamula, B Kiran Kumar: Writing - Review & Editing; Dasari Sreekanth, Survi Mahesh, C.R. Chethan, P.S. Basavaraju, Nagaraju Mukkamula: Visualization.

Funding None.

Data availability Data from this study can be obtained by contacting the corresponding author, Dasari Sreekanth, upon a reasonable request.

Declarations

Competing interest The authors state no identifiable financial conflicts or personal associations that could impact the presented findings.

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