

Biochemical and Physiological Responses of *Capsicum annuum* and Two Competitive Weeds (*Acanthospermum hispidum* and *Euphorbia heterophylla*) Under Induced Salinity

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Abstract

Sweet pepper (*Capsicum annuum* L.) is a widespread and popular crop grown in arid and semi-arid areas and often encounters competition with weeds. This study examined the oxidative stress indicators and antioxidant defense responses of *Capsicum annuum* under induced salinity stress in monoculture and mixed cultures with *Acanthospermum hispidum* and *Euphorbia heterophylla*. Data for the study was obtained from laboratory analysis for physiological and biochemical parameters. Result of the study revealed that Malondialdehyde was significantly affected by treatments ($P < 0.001$), with the highest value content recorded in monoculture at 25% salinity (204.91 ± 4.50) and the lowest in the mixture of *C. annuum* and *A. hispidum* at the same concentration (84.62 ± 3.10). Glutathione was highest in *C. annuum* and *E. heterophylla* mixture at 5% with 35.21 ± 1.70 and lowest at 25% in *C. annuum* and *A. hispidum* mixture (14.80 ± 1.70), with significant treatment effects and interaction with *A. hispidum* ($P < 0.001$). Total protein content showed no significant treatment or interaction effects, although highest values were observed at moderate salinity. Total phenols and flavonoids were significantly affected by salinity levels ($P < 0.001$ and $P < 0.000144$, respectively), with TPH highest in the *C. annuum* and *E. heterophylla* mixture at 10% NaCl with 38.24 ± 1.30 and TF was highest in monoculture at 10% NaCl with 8.92 ± 2.50 . Catalase activity showed no significant differences ($P = 0.057$), whereas superoxide dismutase activity was highly significant ($P < 0.000$), with the highest value at 25% in monoculture with 3.90 ± 0.20 and lowest in the *C. annuum* and *E. heterophylla* mixture with 1.04 ± 0.06 . The study concludes that *Acanthospermum hispidum* have a relatively less stressful effect than *Euphorbia heterophylla* and recommended aggressive management should be encouraged to protect crop physiological health.

Keywords: *Capsicum annuum*, Salinity Stress, Oxidative Stress, Antioxidant Enzymes and Crop-Weeds Interaction.

Introduction

Weeds interference is a serious biotic problem of achieving higher crop yields worldwide and their infestation not only affects the growth and development of cultivated crops but also disrupts their morphological, biochemical and physiological attributes resulting in uneven crop maturity and complicating harvesting operations (Yu *et al.*, 2019). The impact

of weeds on agricultural productivity is staggering, with estimated yield losses amounting to 2.7 million tonnes of grain on global scale (Kumar *et al.*, 2025).

Currently, in developing nations such as Nigeria, Niger, and many countries in the tropics, population growth leads to an increased demand for food, shelter, fibers, and energy and requires both expansion of the area for crops as well as higher crop yields. In both cases, one of the constraints is the occurrence of weed species (Silva *et al.*, 2022). For many decades, Herbicides have been increasingly utilized as a mean of weed control alternative in developing countries. However, the intensive utilization of synthetic chemicals could exacerbate the increased of environmental pollution, herbicide-resistant species, residues in the food chain, health related disorders and the deterioration of soil microbial health (Hasan, 2021). Minimizing synthetic chemical use, choosing low-toxicity chemicals, and wearing protective covering can regulate herbicide toxicity (Darmanti, 2016).

Generally abiotic factors occurring in varying magnitudes will boost weeds invasions globally, but its consequences could vary by region (Jia *et al.*, 2016). As species respond differently to environmental changes such as stress and increased CO₂ that may promote invasion (Vilà, and Weiner, 2004). In addition, stress-tolerant species are already favored in some areas in anticipation of changes triggered by climate change (Lockwood *et al.*, 2013). There is a large consensus in considering crop plants more generally less tolerant to stresses than invasive weeds, thus having a lower plasticity in acclimatizing to environmental changes (Wang *et al.*, 2021). However, Alpert *et al.* (2003), proposed that stress due to salinity, reduced levels of nutrients, water, and light reduce invasive success because (1) exotic plants cannot tolerate the maximum levels of stress to which native species are adapted or (2) in stressful conditions the competitive balance between invasive weeds and crops is altered. Furthermore, Bollen *et al.* (2016), maintained that salinity can exacerbates the replacement of crops by invasive weeds (Legault *et al.*, 2018), but salinity also can also restrain the spread and performance of some weeds (Qi *et al.*, 2017; Liu *et al.*, 2019). Therefore, whether salinity can enhance the invasion of invasive weed species is debatable (Guo *et al.*, 2023). A new model implied that weeds primarily reduce crop yield by altering crop developmental and physiological processes through competition. Multiple studies have reported that stress response pathways are activated when crops such as pepper, tomato and maize are grown in close proximity with weeds during the first 4-8 weeks of growth-the point at which weeds have their greatest negative effect on subsequent crop yields. (Horvath *et al.*, 2023). However, despite growing recognition of these dynamics, there exists a conspicuous gap in the literature regarding the physiological and biochemical mechanisms underpinning crop-weed interactions under stress conditions (Ramegowda *et al.*, 2024). Therefore, this study was carried out to examine the physiological and biochemical response of *Capsicum annuum* and competitive weeds (*Acanthospermum hispidum* and *Euphorbia heterophylla*) under salinity.

Materials and Methods

Study Area

Sokoto State is situated in the extreme northwest of Nigeria, between latitudes 11°30'–15°50'N and longitudes 4°00'–6°00'E, covering approximately 28,232 km². The state shares borders with the Republic of Niger to the north, Zamfara State to the east, and Kebbi State to the south and west (National Bureau of Statistics, 2019). The region falls within the Sudan Savanna ecological zone, characterized by a semi-arid tropical climate with a distinct dry season (November–May) and a brief rainy season (June–October). Mean annual rainfall ranges from 500 mm to 750 mm (Nigerian Meteorological Agency, 2022). Temperatures are high throughout the year, with mean annual temperature of 28.3 °C and maximum daytime temperatures during February–April frequently exceeding 40 °C (Federal Ministry of Environment, 2020). The harmattan winds prevail from November to February, bringing dry, dusty conditions. Vegetation is open savanna woodland dominated by *Acacia* species, *Parkia biglobosa*, and *Anogeissus leiocarpus* (Federal Department of Forestry, 2018). Soils are ferruginous tropical soils with sandy to sandy-loam textures and low organic matter content (Food and Agriculture Organization, 2017). The Sokoto and Rima rivers constitute the major drainage systems (National Water Resources Institute, 2021).

Experimental Site

For this study, a survey was carried out in Kurfi, Hamma' Ali and Yar Barade agroecosystem areas of Sokoto State to determine weed species that are present (invasive weeds) in the area. A total of four species (2 invasive weeds and 1 crop specie) were selected and grown in mono-culture and mixed culture conditions in the Botanical Garden of Kebbi State University of Science and Technology.

Plant Collection and Growing Conditions

The seeds of *Capsicum annuum* were purchased from Sokoto State Central Market, while the invasive weed seeds namely; *Euphorbia heterophylla* and *Acanthospermum hispidum* were surveyed in the Dundaye agroecosystem of Sokoto State. During the survey the weeds were identified locally, collected as (Dan Madaro and Kashin Yawoo) and subjected to identification and authentication by a taxonomist in the herbarium Department of Plant Science and Biotechnology Kebbi State University of Science and Technology Aliero where voucher specimens were deposited at the herbarium. Four (4) plots of 4m² were laid and harrowed in the Botanical Garden for seedbed preparation, both seeds were broadcasted in each plot using a hand. Before the broadcasting weed seeds were presoaked in a 70 °C hot water bath for 4 to 5 min, then dried at room temperature to increase germination rate (Khedir and Roeth, 1981). The plant materials are obtained from the germination of the seeds in the seed bed. After three weeks, When the seedlings developed 3 to 4 true leaves, healthy and uniform seedlings were selected and transplanted into the field laid out of 1m² in a randomized complete block design (RCBD). The treatments consisted of a factorial

combination of one crop and two weed infestation levels arranged in a crops-free and crops-weed mixture each respectively. To mimic salinity sodium chloride (NaCl) was applied to each replicated plot at concentrations of 5, 10, 15, 20, and 25% compositions. The Plants were continuously watered including those from the control treatment. After three consecutive applications of NaCl at intervals of two weeks, five plants from each of the trial plots were randomly selected and tagged avoiding the border rows. These plants were used to measure physiological and biochemical parameters progressively throughout the experiment. The parameters measured are as follows:

Photosynthetic Pigments Determination

Chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids (Caro) were determined following a previously described method (Lichtenthaler, 1987). Fresh leaf material (1g) was crushed with clean mortar and pestle while 20ml of 80% acetone were added to the sample and shaken the homogenates was centrifuge at 5000 rpm for five minutes and transfer the supernatant to a 100ml volumetric flask. The procedure was repeated until the residue is colorless, washed the mortar and pestle with 80% acetone to make the volume of 1000ml with simple solvent and measure the absorbance at 645nm and 663nm against the blank. The following equations were used for the calculation of pigment concentrations (Lichtenthaler and Wellburn, 1983).

$$\text{Chl a (mg/g)} = 12.7 \times (A_{663}) - 2.69 \times (A_{645}) \times \frac{V}{W}$$

$$\text{Chl b (mg/g)} = 22.9 \times (A_{645}) - 4.68 \times (A_{663}) \times \frac{V}{W}$$

$$\text{Caro } (\mu\text{g/mL}) = (1000 \times A_{470} - 3.27 \times [\text{Chl a}] - 104 \times [\text{Chl b}]) / 227$$

Where A = absorbance at specific wavelength

B = final volume of chlorophyll extract in 80% acetone

W = fresh weight of tissue extract

Chlorophylls and carotenoid contents were finally expressed in mg g⁻¹ DW.

Leaf protein (TP) Content Determination

Fifty milligrams (50mg) of leaf tissue were extracted in a buffer solution. Leaf protein content was estimated according to the method of (Bradford, 1976), using bovine serum albumin as a standard.

Estimation of Oxidative Stress Indicator

Malondialdehyde Content Determination: Methanol extracts were prepared by grinding 0.10 g of fresh leaves in a mortar, shaking the samples in a rocker shaker overnight at room temperature, followed by centrifugation at 13,300x g for 15 min. The MDA were quantified in the supernatants, as previously described (Hodges *et al.*, 1999). Each sample was mixed with 0.5% thiobarbituric acid (TBA) that was prepared in 20% trichloroacetic acid (TCA) or with 20% TCA without TBA for the controls and then incubated at 80°C for 15 min in a water bath. The reactions were stopped on ice, and the samples were centrifuged at 13,300xg for 10 min at 4 °C. The absorbance of the supernatants was measured at 532 nm. After

subtracting the non-specific absorbance at 600 and 440 nm, the MDA concentration was calculated by applying the equations that were described by Hodges (Hodges *et al.*, 1999). Based on the molar extinction coefficient of the MDA-TBA adduct at 532 nm.

Non-Enzymatic Antioxidants

Total Phenolic Determination: The TPC was determined with the same 80% methanol extracts that were used for the MDA measurements according to the protocol of Blainski *et al.* (2013), which is based on the reaction with the Folin-Ciocalteu reagent in the presence of NaHCO₃. The reaction mixtures were then be incubated at room temperature in the dark for 90 min, and the absorbance was then be recorded at 765 nm. The TPC concentration was expressed as equivalents of the standard gallic acid (Molina, *et al.*, 2010).

Total Flavonoids Determination

The TF were determined by nitration of catechol groups with NaNO₂, followed by reaction with AlCl₃ under alkaline conditions (Zhishen *et al.*, 1999). The absorbance of the samples was read at 510 nm using catechin as the standard. The TF concentration was expressed as equivalents of catechin (Molina, *et al.*, 2010).

Antioxidant Enzyme Activities

The specific activity of three major antioxidant enzymes, Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione reductase (GR)

One (1g) of leaf materials were crushed in liquid N₂ and mixed with extraction buffer to improve the protein extraction, 1/10 volume of 'high salt buffer' was also added to the samples, mixed well by vortexing, and incubated for 15 min on ice. After centrifugation at 13,500 rpm for 15 min at 5°C, the supernatants were collected, concentrated and centrifuged again to remove precipitated material. The supernatants, referred to as 'protein extracts and was frozen in liquid N₂ and stored in aliquots at -75 °C. The protein concentration in the extracts was measured by the method of Bradford (1976) using the Bio-Rad reagent and bovine serum albumin (BSA) as standard.

The superoxide dismutase (SOD) activity in the protein extracts was determined at 560 nm following the inhibition of nitroblue tetrazolium (NBT) photoreduction in the reaction mixtures containing riboflavin as the source of superoxide radicals (Beyer and Fridovich 1987). A SOD unit was defined as the amount of enzyme that caused 50% inhibition of NBT photoreduction under the assay conditions.

The CAT activity was assessed by the decrease in absorbance at 240 nm, which parallels the consumption of H₂O₂ that was added to the extracts (Aebi, 1984). A CAT unit was defined as the amount of enzyme that decompose one mmol of H₂O₂ per minute at 25 °C (Nakano 1981).

The GR activity was assessed according to Conell and Mullet (1986), following the decrease in absorbance at 340 nm due to the oxidation of NADPH the cofactor of the GR-catalyzed

reduction of oxidized glutathione (GSSG). One GR unit was defined as the amount of enzyme that will oxidize one mmol of NADPH per minute at 25 °C.

Data Analysis

The data obtained was presented as mean and standard deviation obtained result subjected to Two-Way Analysis of Variance (ANOVA). Where treatment was found to be significantly different ($P \leq 0.05$) means separation was carried out using Duncan Multiple Comparison test with SPSS Version 20.0.

Result and Discussions

Table 1 depicts the biochemical parameters of monoculture and mixed culture of *Capsicum annuum* and invasive weeds (*Acanthospermum hispidum* and *Euphorbia heterophylla*) under NaCl concentrations. The results revealed that MDA content was very high in monoculture at 25% concentration with the value of 204.91 ± 4.50 while the lowest value was recorded in the mixture of *Capsicum annuum* and *Acanthospermum hispidum* at 25% concentration with the value of 84.62 ± 3.10 , Malondialdehyde (MDA) content was significantly affected by the treatment ($P < 0.000$), and the interactions with both *A. hispidum* and *E. heterophylla* were also highly significant (Table 1). In Total protein the higher value was recorded in the mixture of *Capsicum annuum* and *Euphorbia heterophylla* at 15% concentration with the value of 0.93 ± 0.08 whereas the lowest value of 0.50 ± 0.08 was obtained at 25% concentration in the mixture of *Capsicum annuum* and *Euphorbia heterophylla* but the total protein (TPR) content did not show any significant treatment or interaction effects (Table 1).

The phenols content was higher at 10% concentration in the mixture of *Capsicum annuum* and *Euphorbia heterophylla* with the recorded value of 38.24 ± 1.30 while the lowest value was also obtained in the mixture of *Capsicum annuum* and *Acanthospermum hispidum* at 25% concentration with the value of 16.40 ± 1.30 . Total Phenolics (TPH) were significantly affected by the treatment ($P < 0.000$), and the interaction with species *A. hispidum* was also significant ($P < 0.000$), while the interaction with species *E. heterophylla* was not significant. The highest value in flavonoids content was observed in the monoculture at 10% concentration with the observed value of 8.92 ± 2.50 in which the lowest value was obtained in the mixed culture of *Capsicum annuum* and *Euphorbia heterophylla* at 25% concentration with the value of 3.11 ± 1.20 . Total Flavonoids (TF) were significantly affected by treatment ($P < 0.000$), but none of the interactions with the invasive species were significance (Table 1).

Table 1 Biochemical Parameters of Mono and Mixed Cultures in Crop-Weeds Interaction

NaCl Conc (%)	Malondialdehyde nmol/g	Protein mg/g	Phenolics (GAE)/ml	Flavonoids (QE)/ml
C. 5	115.38 ± 4.50^d	0.57 ± 0.41^{ab}	20.39 ± 1.41^d	3.11 ± 2.50^{ab}
C. 10	106.83 ± 4.50^c	0.57 ± 0.41^{ab}	41.16 ± 1.41^j	8.92 ± 2.50^{de}
C. 15	98.28 ± 4.50^b	0.69 ± 0.41^{ab}	28.24 ± 1.41^g	4.68 ± 2.50^{abc}
C. 20	94.65 ± 4.50^b	0.69 ± 0.41^{ab}	19.03 ± 1.41^{bcd}	7.44 ± 2.50^{cde}

NaCl Conc (%)	Malondialdehyde nmol/g	Protein mg/g	Phenolics (GAE)/ml	Flavonoids (QE)/ml
C. 25	204.91±4.50 ^j	0.59±0.41 ^{ab}	17.50±1.41 ^{abc}	8.65±2.50 ^{de}
C and A 5	125.38±3.10 ^e	0.84±0.08 ^b	27.34±1.30 ^{efg}	7.50±1.20 ^{cde}
C and A10	98.87±3.10 ^b	0.82±0.08 ^b	26.16±1.30 ^{efg}	7.44±1.20 ^{cde}
C and A 15	94.13±3.10 ^b	0.65±0.08 ^{ab}	25.74±1.30 ^{ef}	6.22±1.20 ^{bcd}
C and A 20	94.65±3.10 ^b	0.62±0.08 ^{ab}	19.43±1.30 ^{cd}	4.68±1.20 ^{abc}
C and A 25	84.62±3.10 ^a	0.59±0.08 ^{ab}	16.40±1.30 ^a	4.12±1.20 ^{ab}
C and E 5	97.41±3.10 ^b	0.77±0.08 ^{ab}	25.65±1.30 ^{ef}	8.92±1.20 ^{de}
C and E10	110.13±3.10 ^{cd}	0.61±0.08 ^{ab}	38.24±1.30 ^{hi}	8.65±1.20 ^{de}
C and E 15	99.58±3.10 ^b	0.93±0.08	27.37±1.30 ^{efg}	4.68±1.20 ^{abc}
C and E 20	94.65±3.10 ^b	0.58±0.08 ^{ab}	19.53±1.30 ^{cd}	7.44±1.20 ^{cde}
C and E 25	84.91±3.10 ^a	0.50±0.08 ^{ab}	16.60±1.30 ^a	3.11±1.20 ^{ab}

The values are presented as mean ± SD of their replicates. Values having different superscripts are significantly different ($p < 0.05$). Keys: C = *Capsicum annuum* in Monoculture; C and A= *Capsicum annuum* and *Acanthospermum hispidum* in Mixed culture; C and E = *Capsicum annuum* and *Euphorbia heterophylla* in Mixed culture.

In Table 2. The superoxide dismutase content was higher in the monoculture at 25% concentration of monoculture with the recorded value of 3.90 ± 0.20 while, the lowest value was also obtained in mixture of *Capsicum annuum* and *Euphorbia heterophylla* at 25% concentration with the recorded value of 1.04 ± 0.06 and its significantly different. However, (SOD) activity was highly affected by the treatment ($P < 0.000$) with both interactions with species *A. hispidum* ($P < 0.001$) and *E. heterophylla* ($P = 0.028$) were also significant.

The catalase content was higher in different concentrations of both monoculture and mixed culture with the recorded value 1.28 ± 0.32 while the lowest value of (0.86 ± 0.08) was also recorded in the mixture of *Capsicum annuum* and *Euphorbia heterophylla* at 25% concentration. Catalase (CAT) showed a non-significant treatment and interaction effect ($P > 0.05$).

Moreover, the glutathione was relatively higher in the mixture of *Capsicum annuum* and *Euphorbia heterophylla* at 5% concentration with the value of 35.21 ± 1.70 while the lowest value was recorded in the mixed culture of *Capsicum annuum* and *Acanthospermum hispidum* at 25% concentration with the value of 14.80 ± 1.70 . Glutathione (GSH) was significantly affected by the treatment at $P < 0.000$ with a significant interaction for *Acanthospermum hispidum* ($P < 0.000$), and non-significant with *Euphorbia heterophylla* at $P > 0.05$ (Table 2).

Table 2 Antioxidant Enzyme Activity of Mono and Mixed Cultures in Crop-Weeds Interaction

NaCl Conc (%)	Superoxidedismutase	Catalase	Glutathione Reductases
C. 5	1.04 ± 0.20^a	1.28 ± 0.32^{bc}	33.71 ± 1.30^{hi}
C. 10	1.76 ± 0.20^{bc}	1.11 ± 0.32^{ab}	32.26 ± 1.30^h
C. 15	1.93 ± 0.20^{cd}	0.98 ± 0.32^{ab}	31.21 ± 1.30^h

NaCl Conc (%)	Superoxidedismutase	Catalase	Glutathione Reductases
C. 20	2.93±0.20 ^f	1.15±0.32 ^{ab}	28.07±1.30 ^g
C. 25	3.90±0.20 ^{ij}	0.86±0.32 ^a	24.80±1.30 ^{ef}
C and A 5	2.16±0.06 ^d	1.28±0.08 ^{bc}	25.71±1.70 ^{fg}
C and A10	3.60±0.06 ^h	1.11±0.08 ^{ab}	22.56±1.70 ^{de}
C and A 15	1.93±0.06 ^{cd}	1.15±0.08 ^{ab}	21.51±1.70 ^d
C and A 20	1.76±0.06 ^{bc}	0.98±0.08 ^{ab}	18.07±1.70 ^c
C and A 25	1.56±0.06 ^b	0.86±0.08 ^a	14.80±1.70 ^b
C and E 5	3.37±0.06 ^g	1.28±0.08 ^{bc}	35.21±1.70 ⁱ
C and E10	1.76±0.06 ^{bc}	1.11±0.08 ^{ab}	33.16±1.70 ^{hi}
C and E 15	1.93±0.06 ^{cd}	0.98±0.08 ^{ab}	32.21±1.70 ^h
C and E 20	2.93±0.06 ^f	1.15±0.08 ^{ab}	26.27±1.70 ^{fg}
C and E 25	1.04±0.06 ^a	0.86±0.08 ^a	22.30±1.70 ^{de}

Figure 1 and 2 depicted that chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids (Caro) show a decreasing trend with increasing NaCl concentration, with the highest and least value recorded in Chl a as in monoculture at 5% and 25% with the value of 3.69±0.12 and 0.44±0.12 in Chl b respectively but chlorophyll a content was affected by the treatments, with an F-value of 700.463 and P-value of < 0.000. with significant interaction effect with both weed species while Chl b, have similar trend with Chl a in which the highest and least value was also recorded in monoculture at 5% and 25% with the value of 3.87±0.11 and 1.31±0.11. However, chlorophyll b was also significantly affected by treatment at (P < 0.000) while the interaction with both weed species were also significant (P < 0.000).

The carotenoids content (figure 3) was also higher in monoculture at 5% concentration with the value of 120.83±0.08 while the least value of 4.35±0.03 was also recorded in the mixed culture of *Capsicum annuum* and *Euphorbia heterophylla* at 25% concentration but the carotenoid content was significantly affected by treatments at (P < 0.001) with strong interaction effect with *A. hispidum* and *E. heterophylla* (P < 0.001). This might occur as 5% monoculture cells receive optimal light and nutrients maximizing potential for carotenoid synthesis and mixed culture at 25% concentration increased shading, nutrient limitation and interspecies interactions will suppress carotenoids production.

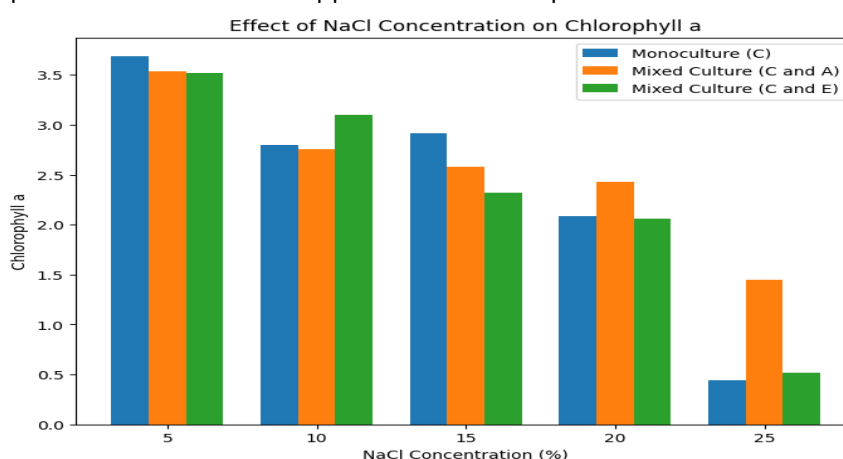


Figure 1 Chlorophyll a Content of Mono and Mixed Culture in Crop-Weeds Interaction

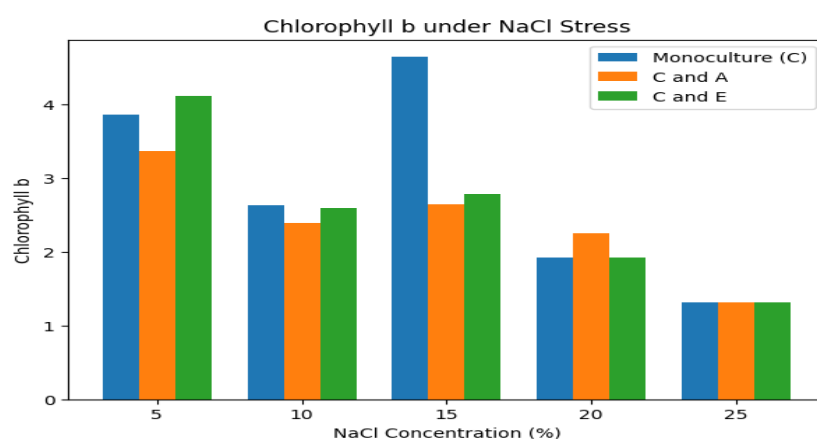


Figure 2 Chlorophyll b Content of Mono and Mixed Culture in Crop-Weeds Interaction

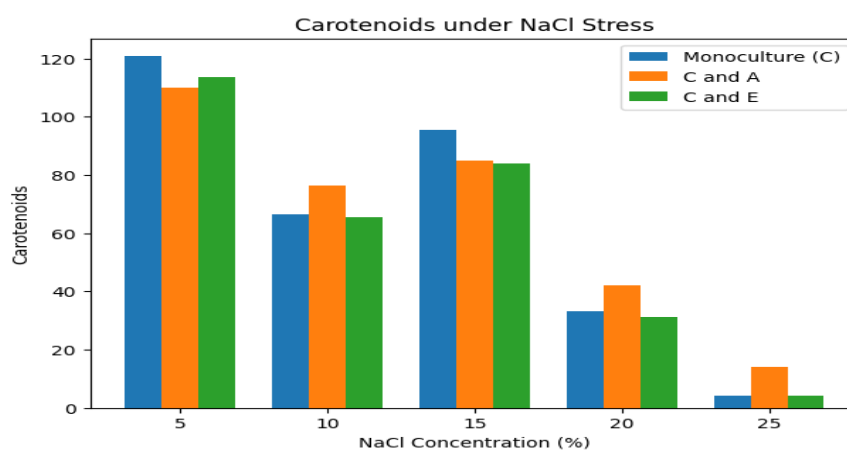


Figure 3 Carotenoids Content of Mono and Mixed Culture in Crop-Weeds Interaction

Discussions

The significant increase in MDA content at higher NaCl concentrations indicates enhanced lipid peroxidation and oxidative damage under salinity stress. High NaCl concentrations disrupt cellular ionic balance and induce oxidative stress leading to membrane damage and increased MDA production. The lower MDA levels observed in mixed cultures especially with *Acanthospermum hispidum* may indicate a stress-moderating effect arising from differential resource utilization under competitive interactions. Similar findings were reported by Hasanuzzaman *et al.* (2023) and Ahmad *et al.* (2022), who observed increased oxidative damage under salinity stress. Similarly, Liu *et al.* (2023), reported that combined biotic interactions could modulate oxidative stress responses under abiotic stress conditions. These findings suggest that oxidative damage in *Capsicum annuum* is influenced not only by salinity intensity but also by interspecific interactions.

The non-significant treatment and interaction effects observed in total protein content suggest that protein levels in *Capsicum annuum* remained relatively stable across salinity gradients. This stability may be associated with the synthesis of stress-related proteins that compensate for protein degradation under saline conditions. Similar non-significant

responses of total protein under salinity stress have been reported in pepper and tomato where protein composition rather than total protein content was more responsive to salt stress (Iqbal *et al.*, 2022).

The increase in phenolic content at moderate salinity levels indicates an adaptive response to oxidative stress. Phenolic compounds are important non-enzymatic antioxidants that scavenge reactive oxygen species (ROS) and protect cellular structures under stress conditions. The significant reduction in phenolic content at higher NaCl concentrations may be due to metabolic impairment and diversion of energy away from secondary metabolite synthesis. Similar observations were reported by Ahmad *et al.* (2022) and Hasanuzzaman *et al.* (2023), who noted enhanced antioxidant activity under moderate salinity but reduced biosynthesis under severe stress conditions.

The significant effect of salinity on flavonoid accumulation further demonstrates the role of flavonoids in antioxidant defense mechanisms under saline conditions. Increased flavonoid accumulation at moderate salinity levels agreed with the findings of Kumar *et al.* (2023), who reported enhanced flavonoid biosynthesis as part of plant adaptation to oxidative stress. However, severe salinity stress reduced flavonoid content due to inhibition of key biosynthetic enzymes and overall metabolic disruption as reported by Wang *et al.* (2024). The absence of significant interaction effects suggests that flavonoid accumulation in *Capsicum annuum* is mainly regulated by salinity stress rather than competition with invasive weed species. This observation agreed with Ahmad *et al.* (2022), who reported that flavonoid synthesis is more responsive to abiotic stress intensity than to biotic interactions. The increase in superoxide dismutase (SOD) activity under high NaCl concentrations unravels the activation of antioxidant defense mechanisms in response to oxidative stress. SOD serves as the first line of defense against reactive oxygen species (ROS) by catalyzing the conversion of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2). The elevated SOD activity observed under severe salinity stress reflects enhanced ROS generation induced by ionic and osmotic imbalance. Similar increases in SOD activity under salinity stress have been reported in pepper, rice and tomato where antioxidant enzyme systems were stimulated to minimize oxidative damage (Hasanuzzaman *et al.*, 2023; Wang *et al.*, 2024). The significantly lower SOD activity in mixed cultures with *Euphorbia heterophylla* suggests that weed competition may suppress the antioxidant defense capacity of *Capsicum annuum* under saline conditions. This reduction may result from competition for nutrients, water and space thereby limiting the availability of essential nutrient suitable for enzyme synthesis. Liu *et al.* (2023), in his study showed that combined biotic and abiotic stresses reduced antioxidant enzyme activities more severely than salinity stress alone.

Catalase (CAT) plays an important role in detoxifying hydrogen peroxide into water and oxygen thereby preventing oxidative injury within plant cells. The non-significant treatment and interaction effects observed in CAT activity suggest that catalase may be relatively stable and less sensitive to salinity stress in *Capsicum annuum* compared with other antioxidant components such as SOD and GSH. Similar non-significant responses of CAT activity under salinity stress have been reported in pepper and cucumber where CAT

activity remained relatively constant while other antioxidant enzymes showed substantial changes (Iqbal *et al.*, 2022; Kumar *et al.*, 2023).

The significant variation in glutathione (GSH) content indicates the important role of glutathione in maintaining cellular redox balance under salinity stress. Glutathione is a major non-enzymatic antioxidant involved in ROS scavenging and protection against oxidative damage. The higher GSH levels observed at low salinity of 5% NaCl concentration suggest effective activation of antioxidant defense mechanisms under mild stress conditions. However, the identified reduction in GSH content at higher NaCl concentrations indicates that severe salinity stress may have overwhelmed the glutathione regeneration system resulting in depletion of antioxidant reserves. Similar findings were reported by Ahmad *et al.* (2022) and Hasanuzzaman *et al.* (2023) that observed reduced glutathione levels under severe salt stress due to excessive ROS accumulation and impaired antioxidant regeneration pathways.

The reduction in chlorophyll a and chlorophyll b contents with increasing NaCl concentration indicates that salinity stress adversely affects the photosynthetic apparatus of *Capsicum annuum*. Salinity stress disrupts chloroplast structure, impairs chlorophyll biosynthesis and accelerates pigment degradation leading to reduced photosynthetic efficiency. The significant decline in chlorophyll pigments observed at higher NaCl concentrations is consistent with findings of (Iqbal *et al.*, 2022; Wang *et al.*, 2024), in rice, tomato and pepper where NaCl stress was associated with impaired photosystem II efficiency and reduced light-harvesting capacity. The similar reduction patterns observed for both chlorophyll a and chlorophyll b suggest that both primary and accessory pigments are equally vulnerable to salinity-induced oxidative damage. Furthermore, the significant interaction effects with both weed species indicate that crop–weed competition may intensify the negative effects of salinity on photosynthetic pigments through competition for nutrients, water and light.

Carotenoids are important accessory pigments involved in photoprotection, scavenging singlet oxygen, and dissipating excess excitation energy generated during stress conditions. The higher carotenoid content observed in monoculture at low salinity at 5% NaCl may indicate favorable physiological conditions that promote pigment synthesis due to adequate nutrient availability and reduced competition. In contrast the severe reduction in carotenoid content observed in mixed culture at 25% NaCl concentration suggests that combined salinity stress and weed competition negatively affected carotenoid biosynthesis. Increased shading, nutrient limitation and interspecific interactions in mixed cultures may further suppress pigment production. Severe salinity stress is also known to disrupt chloroplast metabolism and downregulate carotenoid biosynthetic pathways thereby reducing carotenoid accumulation (Ahmad *et al.*, 2022). Similar reductions in carotenoid content under salt stress have been reported in tomato, maize and pepper, where prolonged salinity exposure caused chloroplast disintegration and reduced antioxidant pigment accumulation (Kumar *et al.*, 2023; Wang *et al.*, 2024). Therefore, the reductions in chlorophyll and carotenoid contents observed in this study indicate that

photosynthetic pigments are highly sensitive physiological indicators of salinity stress and crop–weed interaction effects in *Capsicum annuum*.

Conclusion

This study demonstrated that increasing NaCl concentration significantly alters the physiological and biochemical responses of *Capsicum annuum* grown in both monoculture and mixed culture with *Acanthospermum hispidum* and *Euphorbia heterophylla*. Photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) declined progressively with increasing salinity, with the lowest values consistently recorded at 25% NaCl, indicating impairment of the photosynthetic apparatus under severe salt stress and moderate salinity triggers adaptive antioxidant responses, whereas severe salinity combined with weed competition compromises physiological stability and antioxidant defense mechanisms. Furthermore, photosynthetic pigments, MDA, and antioxidant metabolites can serve as reliable biochemical indicators for assessing salinity tolerance and crop–weed interactions under stress conditions.

Recommendation

Effective control and management of invasive weeds such as *Acanthospermum hispidum* and *Euphorbia heterophylla* should be adopted in agricultural soils in order to minimize competition for nutrients, water, and light thereby reducing stress on *Capsicum annuum*. Cultivation of salt-tolerant varieties of *Capsicum annuum* should be promoted, especially in areas vulnerable to soil salinization to enhance crop survival and productivity under saline conditions. In addition, the application of organic amendments, biofertilizers, and antioxidant-enhancing substances should be considered to improve the antioxidant defense system and reduce oxidative damage caused by salinity stress. Further studies should also be conducted to investigate the physiological, biochemical, and molecular mechanisms involved in crop–weed interactions under salinity stress.

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