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Effects of CO₂ and temperature levels on glyphosate activity and growth of seven weed species

CO₂ ve sıcaklık seviyelerinin glifosat aktivitesi ve yedi yabancı ot türünün büyümesi üzerindeki etkileri

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ABSTRACT

Changes in environmental conditions have a major impact on weed growth and their susceptibility to applied herbicides. We studied the effects of CO₂ and temperature levels on the glyphosate (480 g/l Glyphosate Isopropylamin salt) activity and growth of seven weed species. Three temperature levels (control or normal temperature 26/16 °C (14/10 day/night), normal temperature + 3°C i.e., 29/19 °C and normal temperature + 6 °C i.e., 32/22 °C), and four CO₂ levels (control i.e., 400 ppm, 600 ppm, 800 ppm, and 1000 ppm) were tested. Six doses of glyphosate were: i) ¼, ii) ½, iii) full dose (1440 g a.i./ha), iv) 2-times, v) 4-times, and vi) 8-times of the recommended dose, at 4-6 leaf stage. Generally, the increase in CO₂ and temperature improved weed growth. For most weed species, the most favourable temperature and CO₂ levels were 29 °C and 800 ppm to 1000 ppm. The ED₅₀ (effective dose 50) value for *Echinochloa colonum* (L.) Link., *Amaranthus retroflexus* L., *Amaranthus palmeri* S. Watson, *Portulacaca oleracea* L., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers. and *Physalis angulata* L. showed that some weeds will likely become tolerant to glyphosate with climate change. With increasing temperature and CO₂ concentration, ED value increases, meaning higher herbicide doses are required to control these weeds. As a result, using more herbicides in agricultural areas in the coming years will cause producers to experience more costs and the herbicide resistance problem in weeds will increase to much higher levels.

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INTRODUCTION

Rising carbon dioxide (CO₂) levels in the atmosphere, droughts, global warming, floods and uneven rainfall are important components of climate change. Global warming results from the earth's temperature rising due to a large

increase in population and the burning of fossil fuels, which has added a huge amount of CO₂ and other toxic gases to the atmosphere. It has been observed that the most advanced form of climate change is the enhancement of CO₂

in the atmosphere (IPCC 2020). The current atmospheric concentration of CO₂ is almost 400 ppm, down from below 300 ppm before the global industrial revolution, and it is expected to rise to 700 ppm by the end of this century (IPCC 2020).

These climatic changes can affect different physiological processes in a crop, such as stomatal opening and closing, photosynthetic rate and growth rate (DaMatta et al. 2010, Pernicová et al. 2024). Climate change sometimes negatively impacts crop productivity, reducing crop potential and yields (Asseng et al. 2014, Yang et al. 2024). Some of these climatic changes not only affect the plants but also significantly impact the pest populations of these crops (Elad and Pertot 2014, Jabran et al. 2020, Olesen and Bindi 2002). Elevated CO₂ concentrations can affect various physiological processes in plants and produce a mixed response (both positive and negative) (Misra et al. 2019). C₃ and C₄ species also respond differently to this increase in CO₂ concentration (Hamim 2011). C₃ species respond more quickly and positively than C₄ species because C₄ species are sensitive to CO₂ accumulation (Mooney et al. 1999).

Several invasive weed species have been found to benefit (directly or indirectly) from climate change (Blumenthal and Kray 2014). These weed species develop physiological and morphological adaptations to climate change, allowing them to grow easily and reproduce efficiently as compared to the native plants of the area (Ziska and McConnell 2016). In addition to affecting physiological processes, it also affects weed management programs. The current climate change scenario may favour weeds due to their wide range of environmental tolerances, large dispersal rates, and rapid colonisation (Bajwa et al. 2018), although this prediction may not apply to all weeds (Roger et al. 2015). Weed infestation is one of the most damaging biotic factors. Crop losses due to weeds are more than 30%, which is a higher percentage than crop losses due to diseases and insect pests (Oerke 2006).

Glyphosate is a non-selective broad-spectrum herbicide used to control weeds in cultivated and uncultivated areas. Due to climate change, i.e., increases in temperature and CO₂ concentrations, the effectiveness of glyphosate is largely disturbed. There may be some reasons for this, including increased leaf thickness under elevated CO₂ conditions, which reduces stomata conductance and thus limits leaf uptake of herbicides. This reduction in stomata conductance then reduces the rate of transpiration, which ultimately decreases the herbicide uptake from the soil. Due to the decrease in the absorption rate of herbicide,

the effect of herbicide on plant function decreases causing the ineffectiveness of applied herbicide. However, some literature suggests that the changing climate conditions (warming and rising atmospheric CO₂) will have a neutral effect on the efficacy of herbicides (glyphosate) (Jabran and Doğan 2018). Under current circumstances, it is necessary to determine the effects of applied herbicides on the growth of weeds exposed to changing climate scenarios. Extensive research has been conducted on the impact of climate change on the growth of various crops and weed species as well as on the effectiveness of herbicides against weeds. However, the weeds included in this study have not been previously studied for the impacts of climate change on their growth and the efficacy of herbicides applied to control these weeds. The current study was therefore designed to determine the ultimate effect of climate change on glyphosate activity and weed growth. The effect of temperature and CO₂ on the morphological parameters of C₃ and C₄ weeds was determined. The susceptibility of weeds to glyphosate at high temperatures and CO₂ concentrations was also determined.

MATERIALS AND METHODS

Study site

The study was conducted at the Faculty of Agriculture, Malatya Turgut Ozal University, Malatya, Türkiye. The faculty has a facility of automated growth rooms (5 m × 5 m) where desired CO₂, temperature and humidity levels can be maintained.

Selection of weed species

Seven weed species were selected for this research work (Table 1). Many crops in Türkiye and around the world suffer from these weeds. In addition, they are considered invasive and harmful in agricultural areas (Balah and Balah 2022, Costea et al. 2003, Edmonds and Chweya 1997, Holm et al. 1977, Matzrafi et al. 2023, Rao 2021).

Determining the effect of temperature and CO₂ levels on weed growth

The IPCC (2002) predictions were used to decide the temperature levels in this study. This study had three temperature levels including (i) Control or normal temperature 26/16 °C (14/10 day/night), (ii) normal temperature + 3 °C i.e., 29/19 °C (14/10 day/night), and (iii) normal temperature + 6 °C i.e., 32/22 °C (14/10 day/night). The second and third temperature treatments were considered as medium and high warming.

Four CO₂ levels were tested in this study. (i) Control i.e., 400 ppm, (ii) 600 ppm, (iii) 800 ppm, and (iv) 1000 ppm. The

Table 1. Scientific names, growth habits and families of the weed species used in the experiment.

	Weed species	Growth habit	Family	C ₃ or C ₄
1.	<i>Amaranthus retroflexus</i> L.	Annual, broad-leaved	Amaranthaceae	C ₄
2.	<i>Amaranthus palmeri</i> S. Watson	Annual, broad-leaved	Amaranthaceae	C ₄
3.	<i>Portulaca oleracea</i> L.	Annual, broad-leaved	Portulacaceae	C ₄
4.	<i>Solanum nigrum</i> L.	Annual, broad-leaved	Solanaceae	C ₃
5.	<i>Physalis angulata</i> L.	Annual, broad-leaved	Solanaceae	C ₃
6.	<i>Echinochloa colonum</i> (L.) Link.	Annual, narrow-leaved	Poaceae	C ₄
7.	<i>Sorghum halepense</i> (L.) Pers.	Perennial, narrow-leaved	Poaceae	C ₄

first CO₂ level represents the current CO₂ concentrations on our planet. The other three levels represent the future CO₂ levels as predicted by IPCC (2007).

Experimental materials and setup

Seeds of weed species (Table 1) were collected from the agricultural fields around the Faculty of Agriculture, Malatya Turgut Ozal University, Malatya. The plastic pots used in the study had dimensions of 18 × 18 × 15 cm and a volume of 3.8 liters. These pots were filled with a mixture of compost, sand, and perlite (1:1:1). For each weed species, ten seeds were sown in a pot, and later, a single plant was maintained. Four replications were performed in this experiment using a completely randomized design.

Effect of temperature and CO₂ levels on morphological parameters of weeds

All the weed plants were exposed to the above temperature and CO₂ levels starting from germination until the 8th week of growth. The weeds were then harvested, and growth data were recorded. The data included shoot fresh weight (g), plant height (PH) (cm), root fresh weight (RFW) (g), root length (RL) (cm), plant dry weight (PDW) (g) and root dry weight (RDW) (g). Plant and root dry weights were recorded after drying the relevant parts of the plant in an oven at 65 °C for 48 hours.

Effect of temperature and CO₂ levels on weed susceptibility to glyphosate

Herbicide application is a popular method for controlling weeds, so in this study, the effect of glyphosate on weeds (Table 1) was studied under the established temperature and CO₂ levels. Therefore, weeds grown in greenhouses with different environmental conditions were treated with various doses of glyphosate (480 g/l Glyphosate Isopropylamin salt) herbicide at the 4-6 leaf stage. Six doses of herbicide were applied, including: i) ¼, ii) ½, iii) full dose, iv) 2-times, v) 4-times, and vi) 8-times of the recommended dose. The recommended dose of glyphosate

(480 g/l Glyphosate Isopropylamin salt) was 1440 g a.i./ha. Herbicide applications were carried out with an automatic laboratory sprayer fitted with an 11002 flat-fan nozzle with a spray volume of a pressure of 200 l/ha and a spray pressure of 304 kPa pressure and 5 km/h. First, a stock solution was prepared and diluted to the required concentrations for the herbicide doses. Each herbicide treatment was replicated three times. Herbicide-treated plants were closely monitored, and their dry weight was measured four weeks after glyphosate application.

Dose-response curves were constructed based on the weed biomass obtained after herbicide application on plants grown at each temperature and CO₂ level. Effective doses (ED₅₀ and ED₉₀) were calculated along with the dose-response curves. ED₅₀ (50% control) and ED₉₀ (90% control) values were determined as a measure of the level of control of weeds by the herbicide.

DM reduction (%) data were subjected to a nonlinear regression analysis over herbicide dose using the four-parameter log-logistic model (Knezevic et al. 2007, Ulloa et al. 2011), with the lower asymptote (C) fixed at 0 and the upper asymptote (D) fixed at 100:

$$Y = C + \frac{(D - C)}{\{1 + \exp[B(\log X - \log E)]\}} \quad (1)$$

In this equation, Y is the response (e.g., the percentage reduction in DM), C is the lower limit, D is the upper limit, X is the dose of glyphosate, E is the dose that produces in a 50% and 90% response between the lower and upper limits (also known as the inflection point, I50 or ED₅₀; I90 or ED₉₀) and B is the degree of slope of the slope line.

For the other data, statistical analyses were performed using General Linear Model (GLM) and analysis of variance (ANOVA) using Statistix 8.1 software. Duncan's multiple range test was used to determine the differences among treatments.

Table 2. Effect of temperature levels and CO₂ concentration on morphological parameters of *Amaranthus palmeri* S. Watson and *Amaranthus retroflexus* L.

Variation levels	<i>Amaranthus palmeri</i> S. Watson						<i>Amaranthus retroflexus</i> L.					
	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels												
26°C	35.2 B	12.9 B	4.7 B	0.54 B	1.08 C	0.15 B	28.1 C	23.1 C	4.5 B	0.76 B	1.15 B	0.23 C
29°C	81.3 A	32.9 A	10.9 A	1.42 A	2.95 A	0.56 A	59.9 A	44.4 A	15.8 A	2.84 A	3.34 A	1.05 A
32°C	80.0 A	29.8 A	10.2 A	1.72 A	2.43 B	0.53 A	37.4 B	30.9 B	7.5 B	1.49 B	1.78 B	0.51 B
CO ₂ levels												
400 ppm	61.0 BC	21.1 A	6.6 B	1.01 A	1.80 B	0.36 A	43.9 A	27.7 A	10.1 A	1.54 A	2.45 A	0.56 A
600 ppm	68.4 AB	25.5 A	9.9 A	1.49 A	2.39 AB	0.48 A	40.6 A	35.8 A	7.7 A	1.48 A	1.91 AB	0.52 A
800 ppm	75.1 A	27.9 A	10.9 A	1.36 A	2.51 A	0.43 A	42.6 A	36.7 A	10.3 A	2.01 A	2.43 A	0.69 A
1000 ppm	57.5 C	26.3 A	7.2 B	1.07 A	1.89 B	0.38 A	40.3 A	30.9 A	8.9 A	1.75 A	1.58 B	0.61 A
Temperature × CO ₂												
26°C × 400 ppm	32.5 c	13.9 cde	3.9 d	0.54 a	1.19 de	0.18 b	33.7 de	13.5 d	5.4 b	0.79 bc	1.45 bc	0.26 c
26°C × 600 ppm	31.8 c	9.0 e	4.8 cd	0.51 a	0.92 e	0.09 b	29.7 de	24.8 abcd	4.4 b	0.91 bc	1.15 c	0.27 c
26°C × 800 ppm	38.8 c	16.4 bcde	6.4 bcd	0.64 a	1.25 de	0.15 b	22.9 e	31.6 abcd	4.9 b	0.93 bc	1.09 c	0.26 c
26°C × 1000 ppm	37.7 c	12.3 de	3.7 d	0.49 a	0.97 e	0.17 b	26.2 de	22.3 cd	3.30 b	0.42 c	0.91 c	0.15 c
29°C × 400 ppm	99.1 ab	25.9 abcde	9.7 abc	1.22 a	2.76 abc	0.54 ab	63.4 a	46.3 a	16.5 a	2.47 abc	3.99 a	0.91 abc
29°C × 600 ppm	92.0 ab	37.9 a	14.5 a	2.15 a	3.94 a	0.86 a	54.9 abc	44.1 abc	12.8 ab	2.20 abc	3.28 ab	0.89 abc
29°C × 800 ppm	80.8 b	35.3 ab	12.5 a	1.49 a	3.14 ab	0.49 ab	61.9 ab	44.5 ab	17.2 a	3.50 a	4.15 a	1.28 a
29°C × 1000 ppm	53.1 c	32.5 abcd	7.2 bcd	0.86 a	1.96 bcde	0.34 ab	59.8 ab	42.6 abc	16.8 a	3.19 ab	1.96 bc	1.11 ab
32°C × 400 ppm	51.5 c	23.6 abcde	6.2 bcd	1.28 a	1.47 cde	0.37 ab	34.5 de	23.3 bcd	8.9 ab	1.36 abc	1.89 bc	0.52 abc
32°C × 600 ppm	81.3 b	29.5 abcde	10.3 ab	1.80 a	2.34 bcd	0.49 ab	37.3 cde	38.5 abc	5.9 b	1.34 abc	1.30 c	0.41 bc
32°C × 800 ppm	105.8 a	32.1 abcd	13.7 a	1.93 a	3.16 ab	0.65 ab	43.1 bcd	34.1 abcd	8.8 ab	1.60 abc	2.04 bc	0.53 abc
32°C × 1000 ppm	81.6 b	34.0 abc	10.7 ab	1.85 a	2.76 abc	0.62 ab	34.9 de	27.9 abcd	6.7 b	1.65 abc	1.88 bc	0.57 abc

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

Table 3. Effect of temperature levels and CO₂ concentration on morphological parameters of *Echinochloa colonum* (L.) Link. and *Sorghum halepense* (L.) Pers.

Echinochloa colonum (L.) Link.							Sorghum halepense (L.) Pers.					
Variation levels	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels												
26°C	56.7 C	22.5 B	7.6 A	0.96 B	1.51 A	0.26 B	69.2 C	34.9 B	4.7 B	1.51 B	0.88 B	0.37 C
29°C	72.6 B	34.9 A	9.6 A	1.56 AB	1.93 A	0.74 AB	102.1 B	57.2 A	15.5 A	5.6 A	4.1 A	2.23 A
32°C	93.2 A	36.9 A	10.5 A	3.52 A	2.38 A	1.08 A	133.9 A	60.7 A	12.2 A	5.2 A	3.05 A	1.22 B
CO ₂ levels												
400 ppm	78.3 A	28.7 A	11.8 A	1.71 A	2.57 A	0.74 A	94.0 A	60.7 A	8.1 B	2.18 B	2.06 B	0.91 B
600 ppm	67.7 A	31.1 A	8.3 A	1.73 A	1.78 A	0.59 A	97.3 A	47.5 A	9.7 AB	4.1 AB	2.20 B	1.00 B
800 ppm	74.5 A	31.7 A	8.2 A	1.59 A	1.64 A	0.58 A	116.6 A	45.3 A	15.0 A	6.0 A	3.94 A	2.17 A
1000 ppm	76.3 A	34.3 A	8.6 A	3.01 A	1.78 A	0.83 A	99.1 A	50.3 A	10.5 AB	4.2 AB	2.53 AB	0.99 B
Temperature × CO ₂												
26°C × 400 ppm	65.7 bcde	22.6 b	10.8 a	1.16 b	2.30 a	0.35 b	56.5 d	29.2 b	2.04 b	0.64 b	0.32 b	0.13 b
26°C × 600 ppm	50.4 de	18.7 b	8.7 a	0.75 b	1.79 a	0.19 b	54.0 d	25.3 b	3.3 b	1.2 b	0.41 b	0.18 b
26°C × 800 ppm	45.8 e	30.2 b	4.7 a	1.22 b	0.59 a	0.23 b	85.0 bcd	40.8 ab	5.4 b	1.5 b	0.83 b	0.29 b
26°C × 1000 ppm	65.0 bcde	18.5 b	6.1 a	0.73 b	1.38 a	0.25 b	81.6 cd	44.3 ab	8.0 b	2.6 b	1.96 b	0.85 b
29°C × 400 ppm	80.2 abcd	34.7 ab	9.9 a	1.41 b	2.20 a	0.85 ab	97.0 abcd	81.7 a	12.8 b	3.1 b	3.46 b	1.66 b
29°C × 600 ppm	59.5 cde	39.4 ab	8.0 a	1.41 b	1.78 a	0.82 ab	96.9 abcd	49.1 ab	11.9 b	4.3 b	2.86 b	1.41 b
29°C × 800 ppm	84.0 abc	36.1 ab	14.3 a	2.43 ab	2.94 a	1.01 ab	131.0 abc	51.0 ab	28.3 a	11.8 a	7.7 a	4.89 a
29°C × 1000 ppm	66.7 abcde	29.5 b	5.8 a	0.98 b	0.79 a	0.28 b	83.4 bcd	47.0 ab	9.1 b	3.2 b	2.37 b	0.94 b
32°C × 400 ppm	89.1 abc	28.8 b	14.5 a	2.56 ab	3.19 a	1.04 ab	128.5 abc	71.1 ab	9.3 b	2.8 b	2.41 b	0.95 b
32°C × 600 ppm	93.1 ab	35.1 ab	8.0 a	3.02 ab	1.78 a	0.79 ab	141.0 a	68.0 ab	13.7 b	6.7 ab	3.35 b	1.41 b
32°C × 800 ppm	93.7 ab	28.9 b	5.8 a	1.14 b	1.38 a	0.51 ab	133.8 ab	44.2 ab	11.3 b	4.7 b	3.21 b	1.34 b
32°C × 1000 ppm	97.0 a	54.7 a	13.8 a	7.3 a	3.16 a	1.97 a	132.4 abc	59.5 ab	14.5 b	6.8 ab	3.25 b	1.17 b

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

Table 4. Effect of temperature levels and CO₂ concentration on morphological parameters of *Solanum nigrum* L. and *Physalis angulata* L.

Solanum nigrum L.							Physalis angulata L.					
Variation levels	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels												
26°C	29.4 B	32.4 B	5.8 C	1.85 C	1.48 B	0.49 C	20.8 B	9.7 C	3.9 C	0.39 C	0.34 C	0.05 C
29°C	41.3 A	40.4 A	13.9 A	4.4 B	2.70 A	1.26 A	47.7 A	52.9 A	24.7 A	2.92 B	2.95 A	0.95 A
32°C	32.9 B	46.7 A	8.0 B	5.6 A	1.49 B	0.73 B	50.0 A	36.3 B	20.9 B	6.2 A	2.49 B	0.82 B
CO ₂ levels												
400 ppm	31.9 BC	35.2 BC	9.3 A	3.43 B	1.69 A	0.86 A	34.8 B	25.8 C	10.7 C	1.41 B	1.33 C	0.33 D
600 ppm	36.2 AB	48.5 A	9.5 A	3.96 AB	2.02 A	0.84 A	41.4 AB	40.5 A	17.9 AB	3.79 A	2.09 AB	0.67 B
800 ppm	38.9 A	42.9 AB	9.5 A	4.8 A	1.99 A	0.75 A	44.1 A	34.8 AB	22.5 A	3.80 A	2.64 A	0.89 A
1000 ppm	31.2 C	32.8 C	8.6 A	3.8 AB	1.87 A	0.85 A	37.7 AB	30.6 BC	14.9 BC	3.74 A	1.64 BC	0.52 C
Temperature × CO ₂												
26°C × 400 ppm	27.7 cd	33.8 abcd	6.9 cd	2.86 cde	1.68 bcd	0.75 bcd	27.0 def	6.3 e	4.9 f	0.36 ef	0.39 fg	0.05 d
26°C × 600 ppm	34.0 abcd	37.6 abcd	7.7 bcd	2.19 cde	1.84 bcd	0.48 cd	27.8 def	20.3 de	7.5 ef	0.95 def	0.72 efg	0.12 d
26°C × 800 ppm	31.2 bcd	35.8 abcd	4.7 d	1.62 de	1.35 cd	0.44 cd	11.5 f	3.5 e	0.92 f	0.12 f	0.06 g	0.01 d
26°C × 1000 ppm	24.9 d	22.3 d	3.9 d	0.75 e	1.03 d	0.26 d	17.0 ef	8.7 e	2.28 f	0.16 ef	0.20 fg	0.02 d
29°C × 400 ppm	38.3 abc	38.9 abcd	13.3 ab	3.22 cde	2.13 abcd	1.01 abc	35.8 cde	37.8 c	8.3 def	1.04 def	1.34 def	0.46 c
29°C × 600 ppm	41.9 ab	55.1 a	13.2 ab	3.41 cd	2.83 ab	1.24 ab	51.0 abc	57.0 ab	28.6 b	3.08 cd	3.54 b	1.04 b
29°C × 800 ppm	42.1 a	38.1 abcd	12.5 abc	4.6 bc	2.40 abc	1.28 ab	64.0 a	70.0 a	44.4 a	5.2 bc	5.1 a	1.83 a
29°C × 1000 ppm	42.8 a	29.5 cd	16.7 a	6.5 ab	3.43 a	1.50 a	40.0 bcd	47.0 bc	17.6 cde	2.36 def	1.81 cde	0.46 c
32°C × 400 ppm	29.7 cd	32.8 bcd	7.7 bcd	4.2 bcd	1.26 cd	0.83 bcd	41.8 bcd	33.5 cd	18.9 bc	2.81 cde	2.26 cd	0.48 c
32°C × 600 ppm	32.7 abcd	52.6 ab	7.5 bcd	6.3 ab	1.38 cd	0.79 bcd	45.5 abcd	44.4 bc	17.8 cd	7.4 ab	2.02 cd	0.86 b
32°C × 800 ppm	43.3 a	55.0 a	11.6 abc	8.1 a	2.20 abcd	0.51 cd	56.8 ab	31.1 cd	22.0 bc	6.1 ab	2.78 bc	0.84 b
32°C × 1000 ppm	25.8 d	46.5 abc	5.3 d	4.0 bcd	1.13 cd	0.79 bcd	56.0 ab	36.3 cd	24.9 bc	8.70 a	2.78 bc	1.10 b

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

Table 5. Effect of temperature levels and CO₂ concentration on morphological parameters of *Portulaca oleracea* L.

<i>Portulaca oleracea</i> L.						
Variation levels	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels						
26°C	25.1 B	7.7 B	8.6 B	0.28 B	0.78 B	0.04 B
29°C	34.9 A	15.2 A	28.2 A	0.53 A	2.08 A	0.34 A
32°C	37.4 A	10.4 B	10.9 B	0.20 B	0.73 B	0.05 B
CO ₂ levels						
400 ppm	32.1 A	8.6 B	15.1 A	0.28 B	1.14 AB	0.07 A
600 ppm	34.3 A	11.0 AB	18.5 A	0.43 A	1.45 A	0.09 A
800 ppm	30.9 A	10.1 B	14.5 A	0.28 B	1.04 B	0.06 A
1000 ppm	32.6 A	14.7 A	15.4 A	0.35 AB	1.17 AB	0.35 A
Temperature × CO ₂						
26°C × 400 ppm	29.3 bc	10.2 b	13.4 c	0.43 abc	1.32 bcd	0.06 b
26°C × 600 ppm	27.4 c	8.2 b	10.2 cd	0.38 bcd	1.08 cde	0.06 b
26°C × 800 ppm	17.1 d	5.8 b	4.2 d	0.09 d	0.19 e	0.01 b
26°C × 1000 ppm	26.5 c	6.7 b	6.4 cd	0.21 cd	0.54 de	0.03 b
29°C × 400 ppm	34.3 abc	9.8 b	24.3 b	0.29 bcd	1.57 bc	0.09 b
29°C × 600 ppm	36.7 ab	14.8 ab	34.4 a	0.69 a	2.57 a	0.20 ab
29°C × 800 ppm	34.9 abc	13.9 ab	27.2 ab	0.56 ab	2.18 ab	0.14 b
29°C × 1000 ppm	34.1 abc	22.4 a	26.8 ab	0.56 ab	2.01 ab	0.94 a
32°C × 400 ppm	32.8 abc	6.0 b	7.6 cd	0.12 d	0.52 de	0.05 b
32°C × 600 ppm	38.8 a	10.0 b	10.9 cd	0.22 cd	0.69 cde	0.04 b
32°C × 800 ppm	40.6 a	10.8 b	12.1 cd	0.19 cd	0.74 cde	0.04 b
32°C × 1000 ppm	37.1 ab	15.0 ab	13.0 cd	0.28 bcd	0.96 cde	0.07 b

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

RESULTS

Temperature levels, CO₂ concentrations and their interactions had a significant effect on plant height, shoot fresh weight, and shoot dry weight of *A. palmeri* (Table 2). The root length and root dry weight of *A. palmeri* were affected by CO₂ levels and temperature × CO₂, while CO₂ concentration had no significant effect on root length. The fresh root weight of *A. palmeri* was only affected by CO₂ concentration, while temperature × CO₂ had a non-significant effect. The growth parameters of *A. retroflexus* were only significantly affected by temperature levels and temperature × CO₂ concentration. Shoot dry weight was an exception and was also affected by CO₂ concentration.

At different CO₂ levels, *A. retroflexus* plant height, shoot fresh weight, root fresh weight, root length, and root dry

weight were similar, but these parameters were significantly affected by the temperature levels (Table 2). A temperature of 29 °C was more favourable for the *A. retroflexus* than the other two temperature levels in the study (26 °C and 32 °C). At 29 °C, the weed had the highest plant height, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight compared to the other temperature levels. Moreover, an interaction of 29 °C with different levels of CO₂ was the most favourable for this weed. An interaction of 29 °C × 400 ppm provided the highest plant height and root length of *A. retroflexus*, while 29 °C × 800 ppm provided the highest root fresh weight and root dry weight.

The growth parameters of *E. colonum* were not affected by the CO₂ levels, but the highest temperature level (32 °C) had increased the plant height, root length, root fresh weight and root dry weight of this weed (Table 3). The interaction of the

highest levels of temperature and CO₂ (32 °C × 1000 ppm) provided the highest plant height, root length, fresh root weight and dry root weight.

Temperature significantly affected the growth parameters of *S. halepense* (Table 3). The highest plant height was recorded at 32 °C, while the highest root dry weight was recorded at 29 °C. The *S. halepense* plants grown at 29 °C and 32 °C had a statistically similar (and higher than the plants at 26 °C) root length, shoot fresh weight, root fresh weight and shoot dry weight. Furthermore, the highest shoot fresh weight, root fresh weight, shoot dry weight and root dry weight of *S. halepense* were recorded when these were grown under 800 ppm CO₂. An interaction of 32 °C × 600 ppm CO₂ produced the *S. halepense* plants with the greatest plant height, while 29 °C × 400 ppm produced the greatest root length. Moreover, 29 °C × 800 ppm CO₂ interaction produced the *S. halepense* plants with the highest shoot fresh weight, root fresh weight, shoot dry weight and root dry weight compared to other interaction treatments in the experiment.

A temperature of 29 °C was the most favourable for the growth of *S. nigrum* followed by 32 °C, while 26 °C decreased the weed growth compared to other temperature levels (Table 4). The highest plant height, shoot fresh weight, shoot dry weight, and root dry weight of *S. nigrum* were recorded at 29 °C, while the highest root length and root fresh weight were recorded at 32 °C. CO₂ levels did not affect shoot fresh weight, shoot dry weight and root dry weight of *S. nigrum*, but plant height, root length and root fresh weight of the weed were improved by 600-800 ppm CO₂. The interaction between CO₂ and temperature also had a significant effect, and the temperature level of 29 °C + higher CO₂ levels considerably impacted all growth parameters.

For *P. angulata* weed growth, the temperature of 29 °C was the most favourable followed by 32 °C, while 26 °C decreased weed growth compared to other temperatures (Table 4). While the highest root length, shoot fresh weight, shoot dry weight, and root dry weight were recorded at 29 °C, the highest plant height and root fresh weight were observed at 32 °C. Plant height and root fresh weight were not significantly affected by CO₂ levels, but the shoot fresh weight, the shoot dry weight, and the root dry weight were significantly affected by 600-800 ppm CO₂. The interactive effect of 29 °C × 800 ppm CO₂ produces a higher plant height, shoot fresh weight, shoot dry weight and root dry weight.

The growth of *P. oleracea* was significantly influenced by 29 °C temperature (Table 5). Root length, root fresh weight, shoot fresh weight, shoot dry weight, and root dry weight

were increased at 29 °C, while plant height was increased at 32 °C. CO₂ levels did not affect plant height, shoot fresh weight and root dry weight, while root length, root fresh weight and shoot dry weight were significantly influenced at 600 ppm CO₂. When comparing the interactions, the maximum shoot fresh weight, root fresh weight and root dry weight were obtained by the 29 °C × 600 ppm treatment, while the highest plant height was obtained at the 32 °C temperature and 600 ppm and 800 ppm CO₂ treatments, respectively.

The ED₉₀ value for *E. colonum* was 1816.89 at 26 °C + 800 ppm CO₂, for *A. retroflexus* it was 754.784 at 26 °C + 1000 ppm CO₂, for *A. palmeri* it was 1245.794 at 26 °C + 600 ppm CO₂, for *P. oleracea* it was 1161.96 at 26 °C + 800 ppm CO₂, for *S. nigrum* it was 1307.002 at 26 °C + 600 ppm CO₂, for *S. halepense* it was 370.067 at 32 °C + 400 ppm CO₂ and, for *P. angulata* it was 490.528 at 29 °C + 400 ppm CO₂. Data for ED₅₀ value showed that some of the weeds are likely to become tolerant to glyphosate with climate change. With increasing temperature and CO₂ concentration, the ED₅₀ value increased, which means a higher dose is required to control these weeds (Table 6). The ED₅₀ value (g a.i./ha) for *E. colonum* was 103.55 at 26 °C + 800 ppm CO₂, for *A. retroflexus* it was 47.35 at 29 °C + 800 ppm CO₂, for *A. palmeri* it was 79.64 at 26 °C + 1000 ppm CO₂, for *P. oleracea* it was 109.43 at 29 °C + 1000 ppm CO₂, for *S. nigrum* it was 87.12 at 29 °C + 1000 ppm CO₂, for *S. halepense* it was 37.86 at 32 °C + 1000 ppm CO₂ and, for *P. angulata* it was 18.61 at 29 °C + 800 ppm CO₂.

DISCUSSION

The study showed that weeds were significantly influenced by climate change and developed some tolerance to herbicide use. In the case of *A. retroflexus*, germination was increased by increasing temperature. The higher ED value shows that the efficacy rate of a specific dose of glyphosate decreases, leading to increased tolerance. The previous studies show that maximum germination of *A. retroflexus* was observed at high temperatures (25-35 °C), indicating that the increase in temperature favours its germination and growth (Guo and Al-Khatib 2003, Safavi et al. 2023). Another recent study also showed that the germination rate of *Amaranthus retroflexus* was high at higher temperatures (Khan et al. 2023). Increases in temperature and CO₂ have a positive effect on growth rate. The interactive effect of increased CO₂ and other resources significantly impacts plant height, leaf area, and total biomass of *Amaranthus retroflexus* (Valerio et al. 2011). Herbicide efficacy decreased as CO₂ concentration increased (Ziska et al. 2004) due to

Table 6. Effect of temperature levels and CO₂ concentration on ED₅₀ and ED₉₀ values of weeds

Temperature (°C)	400 ppm			600 ppm			800 ppm			1000 ppm		
	b	ED ₉₀	ED ₅₀	b	ED ₉₀	ED ₅₀	b	ED ₉₀	ED ₅₀	b	ED ₉₀	ED ₅₀
26°C												
<i>Echinochloa colonum</i>	-0.87692	715.46	58.4	-0.79957	963.257	61.701	-0.76697	1816.89	103.55	-1.22955	268.625	44.984
<i>Solanum nigrum</i>	-1.01705	448.155	51.664	-0.62635	1307.002	39.154	-0.67672	933.201	36.298	-0.69589	1016.269	43.227
<i>Amaranthus retroflexus</i>	-1.00584	361.731	40.709	-0.76815	665.312	38.086	-0.74314	678.206	35.262	-0.70468	754.784	33.395
<i>Amaranthus palmeri</i>	-0.91083	1226.93	109.94	-0.75727	1245.794	68.445	-0.72572	1203.331	58.277	-1.60012	314.438	79.649
<i>Portulaca oleracea</i>	-0.37005	113.857	0.30036	-0.84442	200.278	14.845	-0.68187	1161.96	46.316	-0.83516	1041.35	74.99
	400 ppm			600 ppm			800 ppm			1000 ppm		
29°C												
<i>Echinochloa colonum</i>	-0.60356	197.169	5.174	-0.95102	151.732	15.055	-0.9628	226.743	23.143	-3.60138	118.484	64.371
<i>Solanum nigrum</i>	-0.56473	197.4333	4.0336	-1.19545	540.63	86.035	-1.07415	561.8	72.647	-1.26985	491.57	87.12
<i>Amaranthus retroflexus</i>	-1.59867	193.33	48.911	-1.4189	194.552	41.355	-1.5636	193.05	47.357	-1.32548	240.823	45.896
<i>Amaranthus palmeri</i>	-1.0916	588.869	78.678	-1.01745	491.323	56.687	-0.72902	650.087	31.918	-0.61168	218.1201	6.0071
<i>Portulaca oleracea</i>	-1.47499	187.475	42.266	-1.9562	173.712	56.496	-0.96084	338.061	34.345	-2.15839	302.87	109.43
<i>Sorghum halepense</i>	-1.55041	226.454	54.891	-1.15288	235.764	35.057	-1.26954	177.13	31.38	-1.2467	147.688	25.347
	400 ppm			600 ppm			800 ppm			1000 ppm		
32°C												
<i>Echinochloa colonum</i>	-1.08357	166.178	21.874	-1.208	267.75	43.427	-1.01457	503.464	57.734	-2.7578	189.519	85.435
<i>Solanum nigrum</i>	-3.1001	140.6	69.21	-1.13218	375.413	53.911	-1.05765	436.903	54.721	-0.69278	487.579	20.447
<i>Amaranthus retroflexus</i>	-1.2162	201.543	33.096	-0.83672	228.033	16.502	-0.80268	266.147	17.231	-0.64306	341.742	11.215
<i>Amaranthus palmeri</i>	-0.85799	363.358	28.064	-1.0207	310.584	36.082	-1.17656	377.379	58.308	-1.17889	372.331	57.742
<i>Portulaca oleracea</i>	-0.7005	422.005	18.326	-1.9155	139.87	44.418	-0.56705	317.9733	6.6005	-0.54045	230.1461	3.9478
<i>Physalis angulata</i>	-1.00533	199.29	22.403	-0.97986	149.86	15.916	-1.16609	114.72	17.43	-0.74403	135.5574	7.0727
<i>Sorghum halepense</i>	-0.62503	370.067	11.005	-0.70496	328.23	14.54	-0.60005	246.8763	6.3416	-1.46408	169.839	37.868

anatomical changes that affect herbicide uptake rate (Manea et al. 2011). The growth of *A. palmeri* also increased with increasing temperature and CO₂ concentration. Norsworthy et al. (2008) observed that the LD₅₀ for the glyphosate resistant biotype was 2,820 g/ha, which was 79-115 times higher than the LD₅₀ for the glyphosate sensitive biotypes. Furthermore, this amount of glyphosate was more than three times the normal application rate of 840 g/ha. The work of Mohseni-Moghadam et al. (2013) showed that a glyphosate resistant *A. palmeri* had an LD₅₀ of 458 g/ha, which is approximately three times lower than the amount of glyphosate applied in this study to achieve a 50% reduction in weed biomass. Based on the shikimate accumulation and the dose response studies, Brazilian populations of the weed were found to be highly glyphosate-resistant; the herbicide quantity required to reduce the weed growth by 50% was about 4 kg/ha, more than twice the usual application rates (Küpper et al. 2017). For example, *A. palmeri*, an introduced weed in Brazil, is highly resistant to glyphosate, and the dose required to control 80% of the population of this resistant weed is more than 4.5 kg a.i./ha, and this application rate is not economically viable (Carvalho et al. 2015). Sosnoskie et al. (2011) also confirmed multiple resistance in *A. palmeri* to glyphosate and pyriithiobac in Georgia. They observed that 12 and 14-fold higher doses of glyphosate were required to obtain 50% control of *A. palmeri* biotype as compared to the susceptible biotype. Data related to shikimic acid showed glyphosate-susceptible biotypes of *A. palmeri* had shikimate in their leaf tissues after the application of glyphosate, but shikimate was not present in the resistant biotypes (Culpepper et al. 2006). The presence of shikimate in the plants treated with glyphosate indicates that EPSP activity is affected by the herbicide application (Mueller et al. 2003).

In the case of *E. colonum*, a wide range of temperatures exists for germination. It has been observed that *E. colonum* seeds can germinate at 20-34 °C, and 25/15 to 35/25 °C (Peerzada et al. 2016). Recent studies have evaluated some suspected glyphosate resistant *E. colonum* populations in Australia (Werth et al. 2012) and northern California (Alarcón-Reverte et al. 2015). In *S. halepense*, both an increase in temperature and CO₂ levels support the growth parameters. Vila-Aiub et al. (2013) observed that the appearance of glyphosate resistance is strongly temperature-dependent, but a biochemical basis for this dependence is still unknown. Another study shows that high CO₂ levels can flatten and prolong the growth rate of *S. halepense* (Carter and Peterson 1983).

For *P. oleracea*, there was no significant effect of increasing temperature on germination. These results are supported

by Chauhan and Johnson (2009), who found that under laboratory conditions, germination was not influenced by the different temperature levels (35/25 °C, 30/20 °C, and 25/15 °C). Germination of *S. nigrum* was also affected by increasing temperature but started to decrease at higher temperatures. Dong et al. (2020) observed that the germination rate of *S. nigrum* was maximum at 30 °C and started to decrease above 35 °C. *Physalis angulata* germination remained constant at the temperature levels in the study. The response of *P. angulata* showed that at 25 °C and 30 °C temperatures, the germination rate was maximum, while at 40 °C, it started to reduce (Bell and Oliver 1979). Data from different studies show that climate change induces morpho-physiological changes in plants, due to which the uptake and translocation rate of herbicides decreases (Jabran and Dogan 2022, Manea et al. 2011, Ziska et al. 2004). This ultimately causes an increase in the E₅₀ value, which enhances the weeds' ability to tolerate applied herbicides. Consequently, a higher dose of herbicide will be necessary to control these specific weeds. As a result, the increasing use of herbicides in agricultural areas will lead to greater economic costs for producers and a significant rise in weed resistance levels in the coming years.

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The authors have declared no conflict of interest.

Statement of Conflict of Interest

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ÖZET

Çevresel koşullardaki değişikliklerin yabancı otların büyümesi ve uygulanan herbisitlere duyarlılıkları üzerinde büyüktür. Bu çalışmada, CO₂ ve sıcaklık seviyelerinin glifosat (480 g/l Glyphosate Isopropylamin Tuzu) aktivitesi ile yedi yabancı ot türünün büyümesi üzerindeki etkileri incelenmiştir. Üç sıcaklık seviyesi (kontrol veya normal sıcaklık 26/16 °C (14/10 gün/gece), normal sıcaklık +3 °C, 29/19 °C ve normal sıcaklık +6 °C, 32/22 °C) ve dört CO₂ seviyesi (kontrol 400 ppm, 600 ppm, 800 ppm ve 1000 ppm) test edilmiştir. Altı doz glifosat: i) önerilen dozun ¼'ü, ii) ½'si, iii) tam doz (1440 g a.i./ha), iv) önerilen dozun 2 katı, v) 4 katı ve vi) 8 katı, 4-6 yaprak aşamasında uygulanmıştır. Genel olarak, artan CO₂ ve sıcaklık seviyelerinde, yabancı otların büyümesini artırmıştır. Yabancı ot türlerinin çoğu için en uygun sıcaklık ve CO₂ seviyeleri sırasıyla 29 °C ve 800 ppm ile 1000 ppm olarak tespit edilmiştir. *Echinochloa*

colonum (L.) Link., *Amaranthus retroflexus* L., *Amaranthus palmeri* S. Watson, *Portulaca oleracea* L., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers. ve *Physalis angulata* L. için ED₅₀ (etkili doz 50) değeri, iklim değişikliğiyle birlikte bazı yabancı otların glifosata karşı tolerans geliştirme olasılığının yüksek olduğunu göstermiştir. Sıcaklık ve CO₂ konsantrasyonundaki artışla birlikte ED değeri de artmakta olup, bu durum yabancı otların kontrolü için daha yüksek herbisit dozlarına ihtiyaç duyulacağına işaret etmektedir. Sonuçta ileriki yıllarda tarımsal alanlarda daha fazla herbisit kullanımı üreticilerin ekonomik olarak daha fazla masraf yaşamasına ve yabancı otlarda dayanıklılık sorununun çok daha yüksek seviyelere çıkacağına sebebiyet verebilecektir.

Anahtar kelimeler: patlıcan, Şanlıurfa, CaPsol, nested-PCR, moleküler karakterizasyon

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