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Allelopathic Impact of Callistemon Citrinus on Sorghum Bicolor Growth under Salinity Stress

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The current study assessed the impact of 0.09% aqueous leaf extract from *Callistemon citrinus* on the growth of two cultivars of *Sorghum bicolor* L. (Giza 15 - G15 and Dorado - Dor) under two salinity levels (100 mM and 200 mM NaCl). Results revealed that salinity reduced various growth criteria, including insoluble and total carbohydrates, photosynthetic pigments, protein, total phenols, and reduced glutathione, while soluble carbohydrates (sucrose and trehalose) were influenced. Salinity also affected free amino acids (proline and glycine betaine), malondialdehyde, and ascorbic acid. The activity of amylase decreased, while antioxidant enzymes (catalase, peroxidase, superoxide dismutase, ascorbic acid oxidase, and glutathione peroxidase) increased. Additionally, concentrations of K+, Ca++, and Mg++ were reduced, while Na+ accumulated. Application of *Callistemon* extract improved plant growth under stressed or normal growth conditions. The aqueous leaf extract (0.09%) of *Callissstemon sp*., with its enriched phenolic allelochemicals, mitigated the impact of salinity by enhancing photosynthesis, osmoregulation, and the antioxidant defense system of sorghum plants. Furthermore, results indicated that *Sorghum* cultivar G15 exhibited more resistance than Dor, with this resistance correlating with the activity of the antioxidant system.

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1. INTRODUCTION

Allelopathy is defined as direct and indirect effects of allelochemical compounds resulting from organisms which may have inhibitor or stimulator effects on the same or different organism. Allelopathic application could be a natural-based solution, so great attention is given to developing farming techniques, which are sustainable for environment, crop production, protection, and enhancement [1,2]. Some of these allelochemicals possess a good potential to increase the growth and yield of some crops [3]. These compounds affected the equilibrium of plant hormones, absorption of nutrient elements, photosynthesis, respiration, protein synthesis and pigment formation [4] and can be used as bio-herbicide and bio-fertilizers [5].

Salinity is an environmental problem that severely affects both growth and yields in crop plants [6]. It alters many biochemical and physiological processes ranging from protein synthesis to solute accumulation [7], while accumulation of Na^+ leads to ion toxicity that adversely affects plant development [8,9].

To cope with this threat, plants have developed efficient ion transport systems to maintain ion balance within compartments. Additionally, plants generally accumulate several osmolytes, such as proline and glycine betaine (GB), soluble carbohydrates, and amino acids, under abiotic stress, which act as osmotic balancing agents [10,11]. The accumulation of proline and phenolics is dose-dependent. The activity of ascorbate peroxidase (APX) and polyphenol oxidase (PPO) increases, while root and shoot dry weights decrease.

Some studies have examined the effects of stress on plants and traced the interaction between this stress and other allelochemicals. For example, in a study examining the effect of luteolin, a bioactive flavones compound extracted from *Cichorium endivia* L. subspecies *divaricatum*, mitigated salinity impact on two cultivars (Giza 2 and single cross 10) of maize seedling [3]. Similarly, [12] studied the effect of salinity and allelopathic extract of *Amartanthus Retroflexus* and *Chenopodium album* weeds on growth and production of effective medicinal substances in *Valeriana officinalis*. Also, [13] investigated the employment of the aqueous allelopathic extracts of sunflower in improving salinity tolerance of rice. Nevertheless, it is becoming increasingly clear that allelochemicals can modify the response of plants to salinity stress, with many studies indicating that applied allelopathic potential reduced the harmful effects of these stresses on plant growth. Plant physiologists applied many strategies to solve this problem such as hormonal; mineral; osmoprotectant; antioxidant and phenolic treatments, seed priming and allelochemical application [14].

Sorghum is one of the most important cereal crops grown in Egypt, Africa, Asia, America, and Australia [15]. It is an annual tropical C4 plant that grows in semiarid and arid tropical regions [16]. It is used as food, fodder, and in various industries such as the pet food industry and beverages. However, this crop is highly susceptible to drought and soil salinity [17,18].

The objective of this study was to evaluate the allelopathic impact of *Callistemon citrinus* on the growth and some physiological activities of two cultivars of *Sorghum bicolor* grown under salinity stress.

2- MATERIALS AND METHODS

2.1 Growth Experiment

Grains of sorghum (*Sorghum bicolor* L.) were generously provided by the Cereal Research Institute (CRI), Agricultural Research Center (ARC), Giza, Egypt. Two cultivars were selected: Giza 15 (G15) as a long stem variety and Dorados (Dor) as a short stem variety. *Callistemon citrinus* was collected and identified by Walaa Azmy Gabr, Lecturer of Plant Taxonomy, Botany, and Microbiology Department, Faculty of Science, Beni-Suef University.

A preliminary experiment was conducted to determine the moderate and severe salinity doses using the vigor index parameter as described by [19]. The vigor index was calculated as follows:

> Vigor Index = seedling length (mm) × % of germination

Based on the results of this vigor index, 100- and 200-mM sodium chloride were selected for moderate and severe salinity stress, respectively. Another preliminary experiment was performed

to determine the optimal concentration of *Callistemon* extract to be used. The crude extract was prepared by collecting fresh, healthy, uniform leaves, which were thoroughly washed with distilled water and then air-dried. The dried leaves were ground and sieved to obtain their powder. 100 g of leaf powder was soaked in distilled water for 24 hours on an orbital shaker (110 rpm). The supernatant was then completed up to 100 ml and used as a stock solution to prepare serial aqueous concentrations. Grains were soaked in each solution for 24 hours before being sown in soil. After 10 days, the vigor index was applied to calculate the optimum dose of stimulating solution for the main experiment, which was found to be 0.09% aqueous extract.

The main experiment employed a randomized factorial design pot experiment conducted at the research station of the Cereal Research Institute (CRI), Agricultural Research Center (ARC), Giza, Egypt, under open field conditions where the temperature ranged from 36±3°C during the day to 24±2°C at night. Plants were cultivated in clay-loam soil with the following properties: organic carbon 90.1%, total nitrogen 0.12%, C/N ratio 8.3, total phosphorus 0.072%, and calcium carbonate ($CaCO_3$) 3.4%. Sodium content was negligible. The soil was air-dried and then sieved through a 2 mm sieve. Five kilograms of soil were dispensed into porous-bottom black plastic pots (40 cm diameter × 50 cm height) for cultivation. For each sorghum variety, grains were divided into two groups: the first group was soaked for 24 hours in distilled water, while the second group was soaked in the aqueous extract (0.09%). Nine soaked grains of each group were germinated in each pot, and then each group was subdivided into three subgroups. The first subgroup was irrigated with distilled water (as a control), the second one was irrigated with 100 mM NaCl, and the third one was irrigated with 200 mM NaCl. Irrigation was applied every 48 hrs to prevent water stress and ensure proper drainage (Bar-Tal et al., 1991). Seedlings were harvested after 21 days, and various growth criteria (percentage of germination, root and shoot lengths, shoot/root ratio, shoot and root fresh and dry weights, vigor index, and seedling water content) were determined.

2.2 Biochemical Analyses

2.2.1 Estimation of phenolics

Total phenols were measured using the Folin-Ciocalteu reagent [20]. For screening phenolic and flavonoid constituents, an HPLC method was applied as described by [21]. Extraction and hydrolysis were carried out by incubating freeze-dried leaf samples for 16 hours at 35°C in 50% methanol and 1.2 M HCl. Separation of the hydrolyzed phenolics was achieved on an ODS-Hypersil column using a ternary solvent system (dihydrogen ammonium phosphate, orthophosphoric acid, and acetonitrile) with increasing hydrophobicity and changing PH. This experiment was conducted at the Insecticide Laboratory of the National Organization for Drug Control and Research (NODCAR) in Giza, Cairo.

2.2.2 Estimation of sugars content and photosynthetic pigments

Soluble, insoluble, and total carbohydrates were estimated by reaction with cupric ions and arsenomolybdate reagent [22]. Sucrose was calorimetrically estimated using the method described by [23], while trehalose was assayed by its conversion to glucose with trehalase, measured using a glucose assay kit [24]. Amylase (3.2.1.1) was extracted using sodium acetate buffer (pH 5.5), following the method outlined by [25], and then assayed using non-solubilized starch, as per the method described by [26]. Photosynthetic pigments were extracted using 85% acetone [27], and their measurement was conducted under different wavelengths, following the protocol outlined by [28].

2.2.3 Assay of protein, free amino acid, proline, malondialdehyde, ascorbic acid and reduced glutathione

Protein was investigated using the Folin reagent method as described by [29]. Free amino acids (AA) were estimated by reaction with ninhydrin reagent [1% ninhydrin in 0.5M citrate buffer (pH 5.5): pure glycerol: 0.5M citrate buffer (pH 5.5) in a ratio of 5:12:2, respectively], as outlined by [30]. Proline was estimated using the ninhydrin application method according to [31], while glycine betaine (GB) was estimated using potassium tri-iodide solution, following the method described by [32]. Malondialdehyde (MDA), ascorbic acid (AsA), and reduced glutathione (GSH) were estimated using the methods outlined by [33,34,35], respectively.

2.2.4 Assay of antioxidant enzymes

Catalase (CAT, EC 1.11.1.6) was assayed by application of the method of [36]. Peroxidase (POX, EC 1.11.1.7) was assayed by application

the protocol published by [37]. Superoxide dismutase (SOD, EC 1.15.1.1) was assayed by the method of [38]. Ascorbic acid oxidase (ASO, EC 1.10.3.3) activity was calibrated by using the method of [39], while glutathione peroxidase (GPX, EC 1.11.1.9) activity was determined according to the method of [40].

2.2.5 Assay of minerals content

The concentrations of K^+, Na^+, Ca^{++} and Mg^{++} were estimated using flame photometer [41]. This elemental determination was carried out in Occupational Health Department, National Institute of Occupational Safety and Health (NIOSH), Cairo, Egypt.

2-2-6 Statistical analysis

Data were analyzed by one- and two-way analysis of variance (ANOVA) and the significant differences among samples were determined by Duncan's multiple range test at p=0.05 using SPSS v 20. All values were expressed as means of replicates (at least n=3) ± SE

3. RESULTS

Data from Table (1) revealed that the aqueous extract of *Callistemon* leaves contained 11 phenolic compounds, including p-coumaric acid, catechol, chlorogenic acid, coumarin, vanillin, caffeic acid, cinnamic acid, gallic acid, salicylic acid, syringic acid, and ferulic acid (in descending order), with the highest phenolic compound being p-coumaric acid, recorded at 7412.2 µg-1 DW. Additionally, the detected flavonoids were rutin, quercetin, catechin, kaempferol, apigenin, and hesperidin (in descending order), with rutin being the highest detected flavonoid at 4.71 µg-1 DW.

Fig. (1) and Table (2) revealed that salinity stress significantly decreased sorghum (G15) germination to 74.3% and 63.6% under mild and severe concentrations, respectively. It also retarded all investigated growth parameters, including root length (11.3 cm and 9.3 cm), shoot length (23.4 cm and 20.6 cm), seedling fresh weight (4.2 g and 3.1 g), and dry weight (0.65 g and 0.48 g) under mild and severe concentrations, respectively. Moreover, the growth parameters of the G15 cultivar exceeded those of the Dor cultivar, indicating its ability to tolerate both medium and severe salinity stress. Application of aqueous *Callistemon* extract mitigated salt stress and improved all germination and growth criteria for both varieties under all growth conditions. Compared to their corresponding salt-stressed plants (at 100 mM and 200 mM), the germination percentage of sorghum (G15) was promoted to 82% and 69%, and both the length of root (12.8 cm and 10.1 cm) and shoot (25.7 cm and 21.4 cm) were enhanced.

While Soluble sugars, including sucrose and trehalose, were accumulated while insoluble carbohydrates declined in salt-stressed sorghum plants (Table 3). Additionally, a significant decrease of amylase activity (about 20-25%) compared to the control was observed. Data revealed a negative impact of salinity on photosynthetic pigments, but the ratio of Chl a / Chl b did not change (Table 4). Treatment with *Callistemon* extract led to an increase in carbohydrate contents either in stressed or unstressed sorghum G15 variety (478 mg g^{-1} Dwt and 584 mg g-1 Dwt, respectively). There was also an obvious enhancement of amylase activity in either stressed or unstressed sorghum plants (Table 3).

Fig. (2) and Table (5) revealed that the application of *Callistemon* extract increased the content of free amino acids, proline, glycine betaine, and proteins in sorghum. *Callistemon* treatment resulted in the accumulation of phenolic compounds in the investigated sorghum cultivars, reaching about 22% and 17% in unstressed and mild-stressed groups, respectively (Fig. 2). The rate of lipid peroxidation in the G15 cultivar, measured as MDA content, showed a significant decrease in response to *Callistemon* extract (Fig. 2). Additionally, there was a significant increase in ascorbic acid content (15% and 18%) and GSH contents in either stressed (100 mM NaCl) or unstressed sorghum cultivars, respectively.

Table (6) shows the positive effect of *Callistemon* aqueous extract on both sorghum cultivars. It activated all antioxidant enzymes, including CAT, POX, SOD, ASO, and GPX, in both stressed and unstressed plants. It is important to note that only the response of antioxidant enzymes of Dor was lower than that of G15, while all other investigated parameters did not achieve such a gap, which may explain the susceptibility of Dor and the resistance of G15. Salinity led to a reduction in estimated minerals; however, they were increased due to the allelopathic influence of *Callistemon* (Table 7).

Table 1. The HPLC analysis revealed the phenolic and flavonoid constituents (as μg^{-1} dry weight) of the aqueous extract of *Callistemon citrinus* leaves

Phenolic compound	Concentration	Flavonoid compound	Concentration
Caffeic acid	96.18±0.19	Apigenin	2.21±0.024
Catechol	1858.45±4.29	Catechin	3.91±0.029
Chlorogenic acid	1530.23±3.25	Hesperidin	1.65±0.020
Cinnamic acid	63.14±0.74	Kaempferol	3.16±0.023
p-Coumaric acid	7412.19±1.25	Quercetin	4.47±0.032
Coumarin	197.0±0.02	Rutin	4.71±0.034
Ferulic acid	27.31±0.21		
Gallic A	45.93±0.62		
Salicylic acid	34.83±0.25		
Syringic acid	27.53±0.24		
Vanillin	194.0±0.09		

Table 2. Two – ways analysis of the interaction effects of *Callistemon citrinus* aqueous extract and salinity stress on some growth criteria of two cultivars of *Sorghum bicolor* (G15 and Dor). Lengths of root and shoot as (cm) and weights as ($g.seedling^{-1}$)

Source	Soaking		Salinity		Cultivar		Soaking*Salinity		Soaking*Cultivar		Salinity * Cultivar		Soaking*Salinity * Cultivar	
	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value
% of germination	1	715.5***	2	6109***	1	579.5***	2	11.9***	1	19.16***	2	269.3***	2	0.22
Root length	1	151.7***	2	717.5***	1	3446***	2	2.59	1	2.43	2	161.9***	2	2.739
Shoot length	1	189.5***	2	1986***	1	13130***	2	157.6***	1	2.86	2	67.8***	2	65.4***
Shoot/root ratio	1	7.25*	2	24.1***	1	192***	2	41.5***	1	0.148	2	88.1***	2	35.8***
Vigour index	1	1421***	2	8742***	1	19970***	2	236.6***	1	78.9***	2	588.8***	2	9.53***
Seedling F Wt	1	182.9***	2	1338***	1	2186***	2	6.84**	1	9.33**	2	20.6***	2	0.541
Seedling dry matter	1	1409.5***	2	8653***	1	19760***	2	234.8***	1	78.3***	2	582.3***	2	9.45***
WC %	1	0.487	2	47.619***	1	286.07***	2	57.7***	1	2.293	2	51.13***	2	20.6***

Table 3. Interaction effects of *Callistemon citrinus* aqueous extract and salinity stress on soluble carbohydrate (Sol), insoluble carbohydrate (Insol), sucrose, trehalose, and total soluble carbohydrate (as mg g^{-1} D Wt) and on amylase activity as (Δ A_{620} g^{-1} F Wt.mon^{-1}) of two cultivars of *Sorghum bicolor* (G15 and Dor)

3-a. One way analysis

Soaking	Salinity level	Variety	Sol. Carb.	Sucrose	Trehalose	Insol carb	Total Carb.	Amylase
H2O	0.00	G 15	55.44± 0.14f	6.35± 0.06d	4.13± 0.04ef	514.22± 0.56ef	569.66± 0.47f	7.51± 0.22ef
		Dor	63.02± 0.26g	6.78± 0.12g	4.40± 0.08f	589.12± 0.41g	652.14± 0.20g	8.63± 0.16g
	100 mM	G 15	73.82± 0.21b	10.21± 0.13b	7.59± 0.19cd	395.20± 0.52c	433.02± 0.37c	6.2± 0.20c
		Dor	76.22± 0.09cd	11.66± 0.06b	8.19± 0.10b	418.34± 0.07d	494.56± 0.15d	6.62± 0.03d
	200 mM	G 15	102.74± 0.09a	14.54± 0.24a	10.21± 0.12a	239.21± 0.10a	341.95± 0.16a	4.95± 0.04a
		Dor	115.24± 0.21b	16.40± 0.25b	11.42± 0.17bc	271.24± 0.28a	386.48± 0.14a	4.73± 0.11a
0.09	0.00	G 15	63.62± 0.28h	7.62± 0.14e	5.44± 0.14h	520.51± 0.36g	584.13± 0.28h	8.91± 0.15g
		Dor	66.64± 0.12i	8.30± 0.17f	5.82± 0.18h	670.72± 0.28h	737.36± 0.35i	9.87± 0.10i
	100 mM	G 15	64.84± 0.17e	8.34± 0.08d	6.26± 0.05ef	413.74± 0.20e	478.58± 0.12e	7.28± 0.07e
		Dor	70.26± 0.07e	10.61± 0.07fg	7.86± 0.04g	368.22± 0.37f	438.48± 0.39f	7.78± 0.14f
	200 mM	G 15	81.02± 0.09bc	11.63± 0.09b	8.46± 0.08bc	306.46± 0.14b	387.48± 0.19b	5.75± 0.05b
		Dor	87.02± 0.22d	14.16± 0.09d	9.84± 0.05de	320.66± 0.28b	407.68± 0.07c	5.79± 0.11b

3-b. Two ways analysis

Source	Soaking		Salinity		Cultivar		Soaking*Salinity		Soaking*Cultivar		Salinity * Cultivar		Soaking*Salinity * Cultivar	
	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value
Sol. Carb.	1	536.4***	2	1091***	1	105.1***	2	82.0***	1	1.232	2	9.15***	2	7.998
Sucrose	1	310.1***	2	299.7***	1	39.3***	2	35.8***	1	0.023	2	2.32	2	1.133
Trehalose	1	240.2***	2	187.9***	1	23.6***	2	2.445	1	0.271	2	12.4***	2	17.0***
Insol carb	1	213.2***	2	659.0***	1	37.15***	2	2.085	1	0.158	2	17.9***	2	0.644
Total Carb.	1	1130***	2	2857***	1	207.8***	2	58.6***	1	1.512	2	42.8***	2	0.82
Amylase	1	211.5***	2	654.3***	1	37.0***	2	2.110	1	0.16	2	17.7***	2	0.63

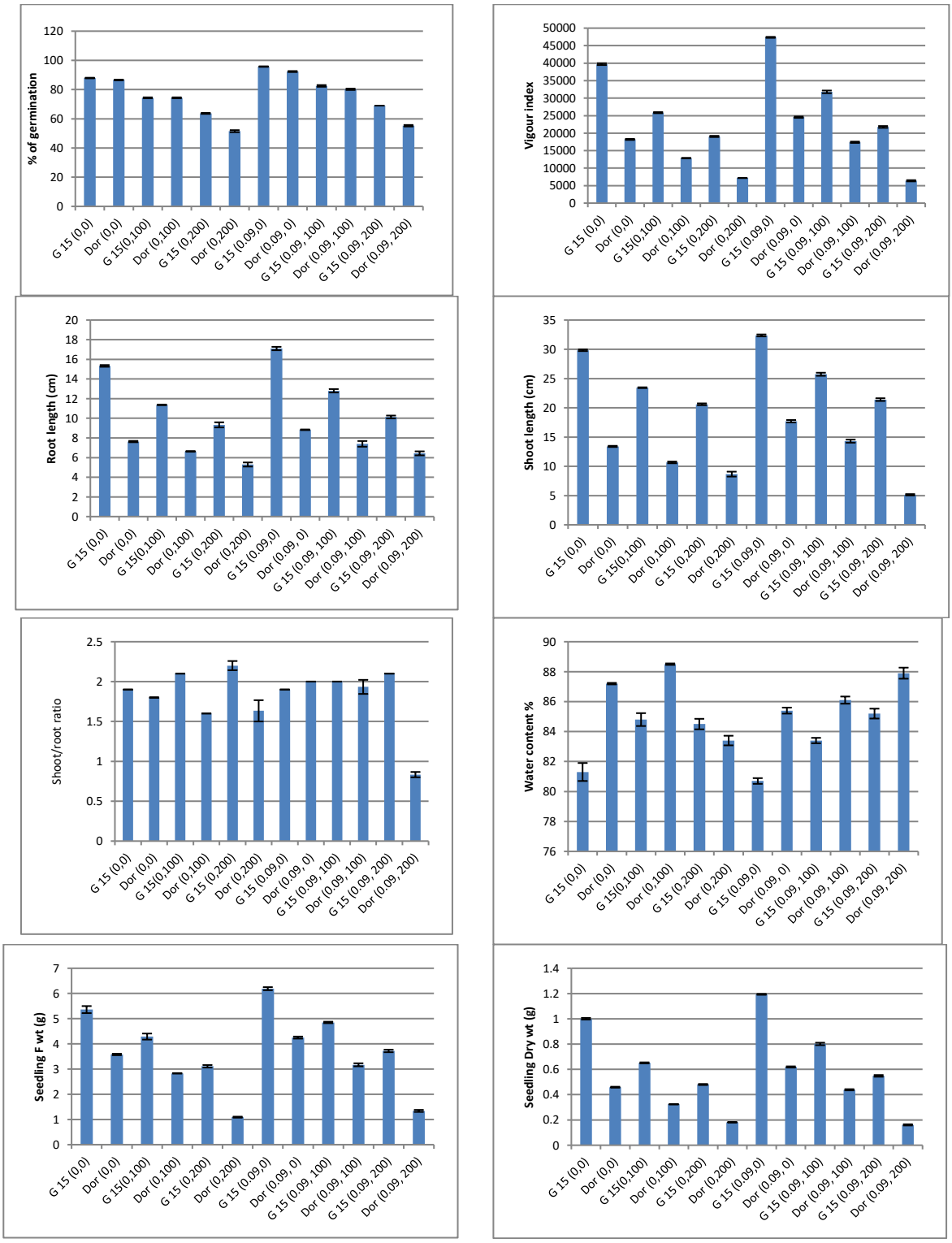

Fig. 1. Interaction effects of *Callistemon citrinus* aqueous extract and salinity stress on some growth criteria of two cultivars of *Sorghum bicolor* (G15 and Dor) (Lengths of root and shoot as (cm seedling^{-1}) and weights as (g seedling^{-1})

Table 4. Interaction effects of *Callistemon citrinus* aqueous extract and salinity stress on chlorophyll a, chlorophyll b, Chl a: Chl b, carotenoids and total pigments (as mg g^{-1} D Wt) of two cultivars of *Sorghum bicolor* (G15 and Dor)

4-a. One way analysis

Soaking	Salinity level	Cultivar	Chl a	Chl b	Chl a /Chl b	Carotenoids	Total pigments
H2O	0.00	G 15	0.41± 0.00e	0.21± 0.00e	1.89± 0.0	0.093± 0.0e	0.71± 0.01f
		Dor	0.45±0.00f	0.24± 0.00f	1.89± 0.0	0.10± 0.0f	0.79± 0.0g
	100 mM	G 15	0.33± 0.00b	0.17± 0.00bc	1.89± 0.0	0.07± 0.0cd	0.57± 0.0c
		Dor	0.35± 0.00c	0.18± 0.00c	1.89± 0.0	0.08± 0.0d	0.61± 0.0d
	200 mM	G 15	0.27± 0.00a	0.14± 0.00a	1.89± 0.0	0.06± 0.0a	0.47± 0.0a
		Dor	0.28± 0.00a	0.15± 0.0a	1.89± 0.0	0.06± 0.0a	0.49± 0.0a
0.09	0.00	G 15	0.49± 0.00g	0.26± 0.00g	1.89± 0.0	0.11± 0.0g	0.87± 0.0h
		Dor	0.55± 0.0h	0.29± 0.00h	1.89± 0.0	0.12± 0.0h	0.97± 0.0i
	100 mM	G 15	0.38± 0.0d	0.20± 0.0d	1.89± 0.0	0.09± 0.0e	0.67± 0.0e
		Dor	0.40± 0.0e	0.21± 0.0e	1.89± 0.0	0.09± 0.0e	0.71± 0.0f
	200 mM	G 15	0.32± 0.0b	0.17± 0.0b	1.89± 0.0	0.07± 0.0b	0.55± 0.0b
		Dor	0.33± 0.0	0.17± 0.0b	1.89± 0.0	0.07± 0.0bc	0.57± 0.0c

4-b. Two ways analysis

Source	Soaking		Salinity		Cultivar		Soaking*Salinity		Soaking*Cultivar		Salinity * Cultivar		Soaking*Salinity * Cultivar	
	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value
Chl a	1	1053***	2	2649***	1	200.0***	2	51.46***	1	3.76	2	41.7***	2	2.38
Chl b	1	480.5***	2	1240***	1	72***	2	26***	1	0.5	2	19.5***	2	2
Carotenoids	1	135.1***	2	362.6***	1	20***	2	8.6**	1	0.8	2	6.2**	2	2.6
Total pigments	1	1158***	2	2924***	1	213.5***	2	59.7***	1	1.48	2	44.0***	2	0.8

Table 5. Two ways analysis of the interaction effects of *Callistemon citrinus* aqueous extract and salinity stress on prol, GB, TAA, protein, TPh, MDA, ASA and GTh of two cultivars of *Sorghum bicolor* (G15 and Dor)

Source	Soaking		Salinity		Cultivar		Soaking*Salinity		Soaking*Cultivar		Salinity * Cultivar		Soaking*Salinity * Cultivar	
	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value	Df	F value
Prol	1	1078***	2	2718***	1	199.8***	2	57.6***	1	1.365	2	41.6***	2	0.679
GB	1	1113***	2	2824***	1	205.6***	2	57.5***	1	1.484	2	42.4***	2	0.854
TAA	1	1098***	2	2830***	1	204.0***	2	59.1***	1	1.653	2	41.4***	2	0.934
Protein	1	1130***	2	2858***	1	208.2***	2	58.8***	1	1.456	2	43.0***	2	0.815
TPh	1	1110***	2	2800***	1	207.2***	2	57.0***	1	1.255	2	41.1***	2	0.883
MDA	1	1183***	2	3076***	1	6631***	2	63.0***	1	47.7***	2	246.3***	2	4.84*
ASA	1	938.13***	2	2257***	1	8150***	2	46.5***	1	59.4***	2	66.2***	2	1.527
GTh	1	1170***	2	3115***	1	18890***	2	63.5***	1	137.6***	2	539.8***	2	10.7***

Table 6. Interaction effects of Callistemon *citrinus* aqueous extract and salinity stress on SOD (as µg-1 FW), CAT, ASO, GPX and POX (all as mM mg protein g^{-1} F Wt) of two cultivars of *Sorghum bicolor* (G15 and Dor).

6-a. One way analysis

Soaking	Salinity level	Cultivar	CAT	POX	SOD	ASO	GPX
H2O	0.00	G 15	12.3± 0.18k	11.2± 0.16k	16.6± 0.24k	85.6± 1.28k	15.1± 0.22k
		Dor	5.11± 0.02e	4.66± 0.03e	6.88± 0.04e	35.3± 0.20e	6.26± 0.03e
	100 mM	G 15	10.0± 0.12i	9.11± 0.13i	13.5± 0.20i	69.1± 0.96i	12.2± 0.17i
		Dor	3.92± 0.02c	3.58± 0.02c	5.31± 0.02c	27.2± 0.14c	4.83± 0.03c
	200 mM	G 15	8.26± 0.06g	7.61± 0.06g	11.1± 0.08g	57.2± 0.43g	10.1± 0.08g
		Dor	3.17± 0.02a	2.89± 0.02a	4.28± 0.03a	21.9± 0.13a	3.89± 0.02a
0.09	0.00	G 15	15.0± 0.11l	13.7± 0.10l	20.3± 0.14l	104.1± 0.74l	18.4± 0.13l
		Dor	6.22± 0.05f	5.68± 0.04f	8.40± 0.07f	43.1± 0.34f	7.65± 0.06f
	100 mM	G 15	11.6± 0.05j	10.6± 0.04j	15.7± 0.06lj	80.9± 0.32j	14.3 ± 0.06j
		Dor	4.56± 0.05d	4.16± 0.05d	6.16± 0.07d	31.6± 0.37d	5.61± 0.07d
	200 mM	G 15	9.61± 0.07h	8.77± 0.06h	12.9± 0.10h	66.5± 0.49h	11.8± 0.09h
		Dor	3.68± 0.01b	3.36± 0.01b	4.97± 0.01b	25.5± 0.07b	4.52± 0.01b

6-b. Two – ways analysis

Source	Soaking		Salinity		Cultivar		Soaking*Salinity		Soaking*Cultivar		Salinity * Cultivar		Soaking*Salinity * Cultivar	
	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value
CAT	1	849.4***	2	2003***	1	21750***	2	40.7***	1	158.2***	2	255.0***	2	5.86**
POX	1	775.0***	2	1832***	1	20140***	2	40.2***	1	140.6***	2	231.3***	2	6.05**
SOD	1	763.3***	2	1794***	1	19550***	2	37.6***	1	141.1***	2	231.4***	2	5.10*
ASO	1	770.9***	2	1805***	1	19600***	2	36.8***	1	142.2***	2	232.7***	2	4.86*
GPX	1	799.7***	2	1872***	1	20340***	2	39.0***	1	147.6***	2	240.7***	2	5.08*

** Significant at p ≤ 0.05, ** p ≤ 0.01 and *** at p ≤ 0.0*

Table 7. Interaction effects of *Callistemon citrinus* aqueous extract and salinity stress on some mineral concentration (as mM mg g^{-1} D Wt) of two cultivars of *Sorghum bicolor* (G15 and Dor)

7-a. One way analysis

Soaking	Salinity level	Variety	K	Na	K/Na ratio	Ca	Mg
H2O	0.00	G 15	14.6± 0.25d	0.38± 0.02bc	38.7± 2.3c	19.1± 0.04h	15.1± 0.03h
		Dor	14.5± 0.02d	0.34± 0.04b	43.6± 5.3c	19.5± 0.02j	15.4± 0.02j
	100 mM	G 15	13.2 ± 0.02bc	1.25± 0.02f	10.6± 0.15ab	12.6± 0.04f	10.3± 0.03f
		Dor	13.8± 1.08cd	1.38± 0.01g	10.0± 0.88ab	12.5± 0.02e	10.2± 0.01e
	200 mM	G 15	9.81± 0.06a	2.30± 0.02i	4.25± 0.06a	7.26± 0.03a	6.43± 0.02a
		Dor	9.66± 0.17a	2.38± 0.03i	4.06± 0.05a	7.57± 0.06b	6.65± 0.04b
0.09	0.00	G 15	16.4± 0.09e	0.24± 0.03a	71.5± 10.6d	19.4± 0.04i	15.3± 0.03i
		Dor	16.3± 0.37e	0.44± 0.03c	37.5± 2.7c	19.8± 0.03k	15.6± 0.02k
	100 mM	G 15	12.6± 0.08b	0.74± 0.03d	17.2± 0.75b	14.4± 0.06g	11.6± 0.05g
		Dor	12.6± 0.13b	0.86± 0.04e	14.7± 0.7ab	14.4± 0.04g	11.6± 0.03g
	200 mM	G 15	10.6± 0.04a	1.45± 0.01g	7.33± 0.07ab	10.2± 0.03c	8.59± 0.02c
		Dor	10.4± 0.05a	1.56± 0.03h	6.67± 0.14ab	10.7± 0.03d	8.96± 0.02d

7-b. Two – ways analysis

Source	Soaking		Salinity		Cultivar		Soaking*Salinity		Soaking*Cultivar		Salinity * Cultivar		Soaking*Salinity * Cultivar	
	Df	F value	df	F value	df	F value	df	F value	df	F value	df	F value	df	F value
K	1	8.58**	2	241.9***	1	0.00	2	15.6***	1	0.38	2	0.45	2	0.17
Na	1	804.3***	2	3204***	1	39.4***	2	215.0***	1	7.61*	2	0.75	2	5.46*
K/Na ratio	1	12.1**	2	155.1***	1	6.98*	2	2.27	1	10.8**	2	4.73*	2	9.07***
Ca	1	5850***	2	72930***	1	124.0***	2	1244***	1	4.87*	2	52.2***	2	1.58
Mg	1	5979***	2	74180***	1	128.0***	2	1264***	1	5.95*	2	53.8***	2	1.74

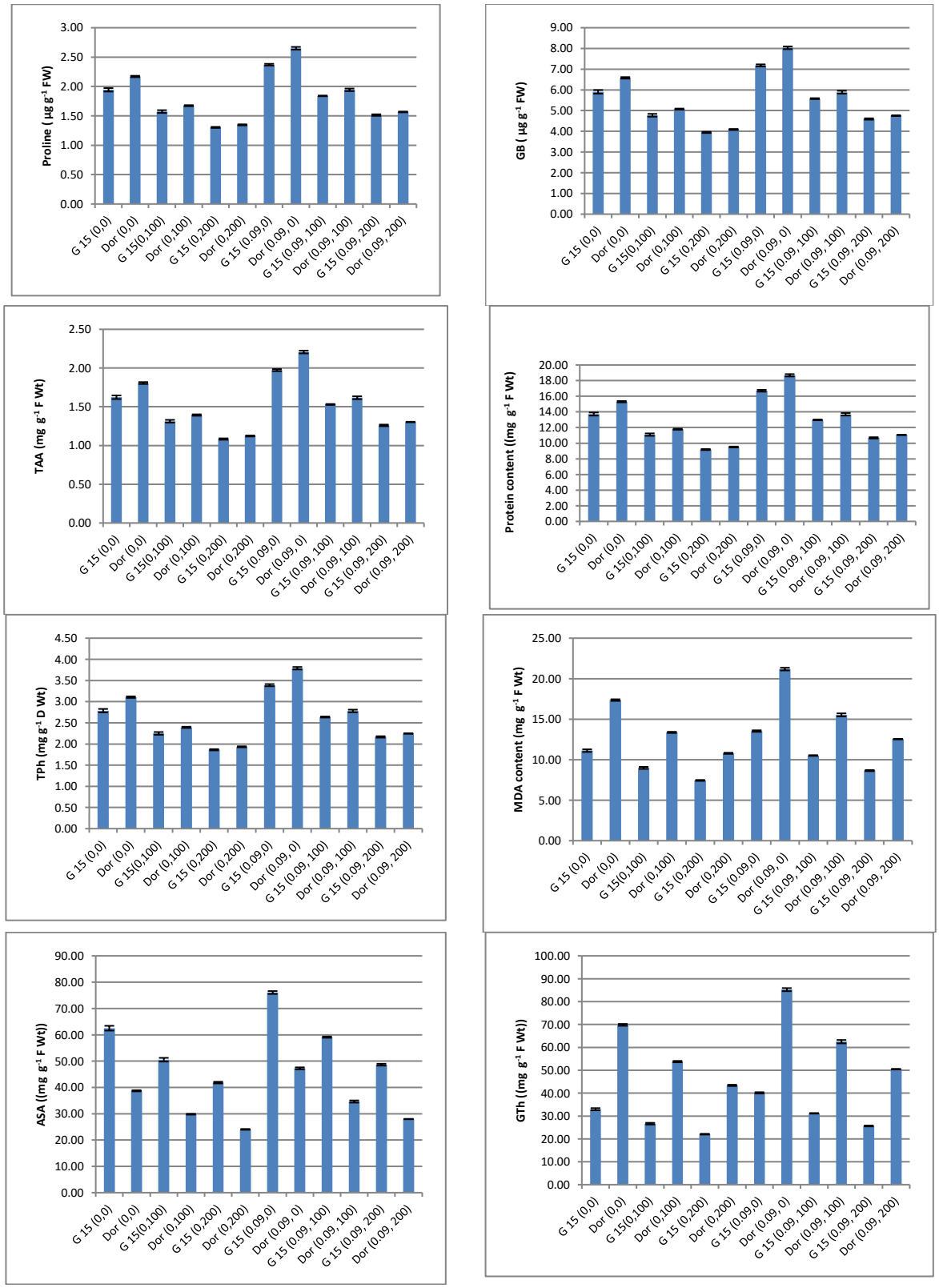

Fig. 2. Interaction effects of *Callistemon citrinus* aqueous extract and salinity stress on prol, GB, TAA, protein, TPh, MDA, ASA and GTh (as mg g^{-1} D Wt) of two cultivars of *Sorghum bicolor* (G15 and Dor)

4. DISCUSSION

The current study demonstrates the harmful effects of salinity on sorghum germination, biomass, and physiological activities. Salt stress reduces plant growth and yield, as plants allocate most of their photosynthetic energy to root production in search of water and/or to reduce water loss, thus maintaining relatively high-water relations [42]. The evaluation of the positive impacts of *Callistemon citrinus* extract, as a bio-stimulant, in alleviating the negative effects of mild and severe (100 and 200 mM NaCl) salt stress on two sorghum cultivars (G15 and Dor) was monitored. The aqueous extract of *Callistemon sp*. increased various growth parameters (seedling length, fresh and dry biomass, and vigor index). A differential response between the investigated sorghum cultivars was observed, with a preference for the G15 cultivar, which exhibited more salt-tolerant behavior. Similarly, naturally occurring plant growth stimulants such as *Moringa oleifera* extract ameliorated salinity-induced adverse effects and enhanced growth and yield of wheat [43,44].

Exposure of sorghum cultivars to salinity stress resulted in an accumulation of soluble carbohydrates, particularly sucrose and trehalose, while retarding insoluble and total carbohydrate contents, and inhibiting amylase activity. Stressed sorghum plants accumulated soluble sugars, which contribute to raising plant osmotic potential [45]. Sucrose, acting as an osmoprotectant, was accumulated under saline stress, attributed to the activity of sucrose synthesis-related enzymes [46]. Similarly, salinity led to an accumulation of trehalose, one of the most effective osmoprotectants under abiotic stress, due to its efficient role as a membrane-stabilizing agent [47]. Trehalose scavenges reactive oxygen species (ROS), mainly H_2O_2, enhances the biosynthesis of some phenolic compounds (antioxidant compounds) and compatible osmolytes, and maintains membrane integrity [48,49]. Our results revealed that the application of *Callistemon* extract caused an increase in total soluble carbohydrates and individual sucrose and trehalose in either stressed or unstressed sorghum plants compared to the untreated control. The significant accumulation of previously mentioned soluble sugars indicates their adjusting role in helping sorghum cultivars mitigate salinity stress.

Apparently, there is a positive effect of *Callistemon* extract on sorghum content of photosynthetic pigments, both chlorophyll and carotenoids, indicating a growth-promoting role if *Callistemon* extract is applied to unstressed crops and its role in helping crops mitigate harsh salinity conditions. *Callistemon* extract increased treated sorghum contents of free amino acids, proline, and glycine betaine, which can be ascribed as tolerance mechanisms against salinity stress [50], acting as a membrane stabilizer [51], and scavengers of reactive oxygen species (ROS) [52]. Glycine betaine effectively stabilizes the quaternary structures of enzymes and complex proteins, and maintains the highly ordered state of membranes at non-physiological temperatures and salt concentrations [53]. Proline may also reduce lipid peroxidation [54], and enhance the activities of some antioxidant enzymes like catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), and ascorbate peroxidase (APX) [55]. Additionally, *Callistemon* extract enhanced the accumulation of the phenolic compounds, which participate in plant resistance against stress factors. Such accumulation was coincident with that of soluble sugars, that may be due to the tendency of plants to accumulate phenolic compounds mostly in glycosides form as a strategy of detoxification [56], besides aglycones which, also, can scavenge the harmful ROS [57], particularly hydroxyl radicals [58].

HPLC analysis revealed that *Callistemon citrinus* is rich in flavonoids, which act as strong antioxidant compounds and play an important role in plant stress tolerance, thereby contributing to crop productivity [59]. Their impact may be attributed to their role in enhancing the antioxidant system by scavenging free radicals and other reactive oxygen species through hydrogen atom and/or electron donation [14]. Additionally, the improving impact of *Callistemon* extract may result from the effect of phenolics (such as cinnamic and benzoic acids) through their anabolic role as precursors of phytohormones and respiration via their signaling role during gene transcription [60].

Treating the studied sorghum cultivars with the aqueous extract of *Callistemon* resulted in the improvement of their antioxidant defense system (both non-enzymatic and enzymatic antioxidants). This was evident in the increased content of glutathione (GSH) and ascorbic acid, as well as the activity of some antioxidant enzymes. Ascorbic acid, acting as a signaling molecule, is involved in the regulation of antioxidant processes such as reacting with O_3

and mitigating photo-oxidative situations [61], while GSH acts as a substrate for glutathione peroxidase (GPX) and glutathione-S-transferases (GST), which are involved in the elimination of ROS [62]. Several plant studies have revealed that the upregulation of the AsA-GSH pathway enzymes and the enhancement of AsA and GSH levels confer plants better tolerance to abiotic stresses and improve osmoregulation, plant water and nutrient status, and overall productivity [63].

Callistemon extract significantly activated all investigated antioxidant enzymes (CAT, POX, SOD, ASO, and GPX). The increase in the activity of antioxidant enzymes may be attributed to their role as scavenging agents for reactive oxygen species (ROS) resulting from salinity stress [64]. Peroxidases (POXs) are a group of isoenzymes capable of scavenging ROS, playing an effective extracellular signal transduction role for stomatal closure and cell elongation [65]. Increasing SOD activity in sorghum is a mechanism to mitigate salt stress conditions by enhancing the ability to eliminate ROS [66]. Additionally, GPX serves several roles, including the regulation of the cell cycle [67], hydrogen peroxide detoxification, signal transduction, and redox sensing [68]. It is involved in various biotic and abiotic stress adaptation pathways via H_2O_2 scavenging, which might use three different reductants: GSH, NADPH, and thioredoxin [69].

The estimation of changes in mineral content either under salinity stress or after *Callistemon* treatment is shown in Table (7). The obtained reduction in the investigated minerals due to salinity stress may be attributed to the adverse effects of drought on nutrient availability, uptake, transport, and accumulation in the tested plants [70]. Such reduction may also be due to a retardation in their uptake activity by plant roots because of the shortage of energy consumed for osmotic adjustment against stress [71], or due to an inhibition of specific proteins responsible for mineral uptake [72].

The increase in the sorghum mineral contents after exposure to *Callistemon* extract may be attributed to activation in plant various metabolic processes and consequently mineral requirements. Such increment may be due to activation of some catalytic enzymes involving mineral absorption [73] and/or to the role of *callistemon* phenolics and flavonoids in maintenance of membrane integrity and enhancement of mineral uptake [71].

5. CONCLUSION

The aqueous leaf extract (0.09%, w/v) of *Callistemon citrinus* L. improved the growth of both tested cultivars (G 15 and Dor) of *Sorghum bicolor* L. under normal conditions or salinity stress up to (200 mM NaCl). Moreover, the tolerance of cv. G 15 was greater than that of cv. Dor, which exhibited a less effective antioxidant enzyme mechanism. Apparently, there is a positive effect of *Callistemon* extract on various physiological activities of sorghum plants, such as photosynthetic pigments (both chlorophyll and carotenoids), osmoregulatory, and antioxidant defense systems, indicating a growth-promoting role when *Callistemon* extract is applied to unstressed crops and also its role in helping crops mitigate harsh salinity conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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