



**PRIMING MAIZE SEEDS WITH *ECHINOCHLOA COLONA* ALLELOPATHIC
AQUEOUS EXTRACT ALLEVIATES SALT STRESS**

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ABSTRACT

Seed priming is among the promising approaches to enhance plant productivity under salt stress. Plant extracts as seed bio-priming agents are currently drawing attention. To test the potentiality of *Echinochloa colona* allelopathic aqueous extract (EAAE) to alleviate maize salt stress, maize grains were primed with distilled water (hydroprimed) and EAAE (1% and 3%). In pot experiment, maize developed from both hydroprimed and EAAE primed seeds were grown at different levels of salt stress (0.0, 100 and 200 mM NaCl). Salinity adversely affected maize growth and significantly declined its shoot and root lengths. In response to salt stress, proline (prol), glycine betaine (GB), free amino acids (AA), total phenolics (TPh) and sodium ions (Na⁺) accumulated in maize leaves, while levels of soluble proteins, total lipids (TL), N and P attenuated. Seed bio-priming with EAAE at lower dose (1%) effectively secured macromolecules and diminished the loss that caused by salinity in soluble proteins and total lipids. Application of 1% EAAE also enhanced the ionic homeostasis and mineral nutrition by increasing K⁺:Na⁺ and Ca²⁺:Na⁺ ratios and increased total P and N contents, in addition to modulating osmoregulation. Thus, the potentiality to mitigate the inhibitory effects of salt stress recommends EAAE to be applied as seed bio-priming agent to enhance salt tolerance of maize.

Keywords: Allelopathic potential; *Echinochloa*; Maize; Salinity; Seed priming

INTRODUCTION

Salinity is one of the factors dramatically affecting agricultural productivity worldwide. More than 20% of the cultivated lands around the world is affected with high levels of salt [33]. Salinity reduces plant growth, inhibits metabolic processes due to their induced osmotic stress, ionic toxicity and oxidative stress. Against salinity-induced osmotic, ionic and oxidative effects, plants develop their osmoregulation and ionic homeostasis mechanisms as well as their antioxidative defense system. The antioxidant defense system includes non-enzymatic antioxidants such as phenolics, ascorbates and glutathione, and the enzymatic ones includes all the enzymes functioning on scavenging reactive oxygen species (ROS). Tolerant plants accumulate proline and glycine betaine (GB) as osmoregulation mechanisms and enhance the activity of their antioxidant enzymes under salt stress [30]. Exclusion of sodium, compartmentation into vacuoles plus limiting sodium translocation to shoot are among the adaptive mechanisms of maize under salt stress [19].

Maize (*Zea mays*), family Poaceae, is one of the most important cereal crops worldwide. Maize can be processed into a variety of food and industrial products, including starch, sweeteners, oil, beverages, glue, industrial alcohol, and fuel ethanol

[46]. Although it's wide intraspecific genetic variation, maize generally is considered as a moderately sensitive crop to salt stress [19]. Many attempts were done to improve salt tolerance of maize through conventional breeding, mutation, genetic engineering, applying arbuscular mycorrhizal fungi and growth promoting bacteria, and seed priming [37, 19].

Farooq *et al.*, (2015) [19] reported that, seed priming was applied to enhance not only germination but also plant growth and yield. Among the priming agents that were investigated, water, sodium or potassium chloride, hormones, hydrogen peroxide (H₂O₂) and ascorbic acid (ASA). Naturally occurring metabolites is a category currently attracting attention as promising priming agents. This category includes osmolytes, hormones, polyamines and antioxidants [48].

Additionally, previous studies reported the enhancement of germination and growth when using plant extract as priming agent [9, 42]. Yasmeeen *et al.*, (2013) [56] attributed the promotive effect of *Moringa oleifera leaf* extract (MLE) as a seed priming agent to its content of antioxidants such as ascorbate, carotenoids, phenols, and flavonoid. The stimulatory effect of priming seeds with plant extract was ascribed in some cases to its allelochemicals [1, 6] though being

concentration dependent.

Allelopathy is the interference to plant growth resulting from chemical interactions among plants and other organisms mediated through release of plant-produced bioactive secondary metabolites referred to as allelochemicals [35]. Allelochemicals are released from plants into the environment through exudation from roots, leaching from leaves and other aerial plant parts, volatile emissions and the decomposition of plant material [55]. To alleviate stress effects on plant, utilizing the allelopathic potential of plant extract in seed priming was attempted [20, 7]. *Echinochloa colona* L., family Poaceae, is a weed competes many crops like wheat, barley and rice, was reported to have allelopathic potency [22]. In this study we investigated the effect of priming seeds of maize with allelopathic aqueous extract. The extract was passed through muslin tissue and then filtered through Whatmann No. 42 filter paper. According to germination (%) in our preliminary experiment (unpublished data), the concentrations 1% (as optimum stimulating dose) and 3% (as minimum inhibitory dose) were selected for priming maize grains in the current study.

Experiment was carried out in a completely randomized factorial design. Maize grains were primed by soaking for 24 hr. in aerated solutions of 1% and 3%

of *E. colona* on the plant growth. The study also discussed the physiological responses of maize primed with *E. colona* extract, as compared with the hydro-primed one, to saltstress.

Materials and methods

Maize (*Zea mays* L. cv TH324) grains were obtained from Agriculture Research Center, Giza, Egypt, while *E. colona* was collected from cultivated fields in Reyad Pasha Valley, Beni-Suef governorate, Egypt. It was identified by taxonomist colleagues, Faculty of Science, Beni-Suef University. *E. colona* leaves were washed with running tap water, then with distilled water and air dried at room temperature. The dried leaves were ground in a grinder and passed through sieves. Ground air-dried leaves were soaked in distilled water for 24 h on orbital shaker (110 rpm).

E. colona allelopathic aqueous extracts (EAAE) and those primed with distilled water (hydroprimed) served as control. Grains were sown at the late of April 2017 in pots (15 × 15 cm) filled with one kg of soil as 10 grains per pot. The soil used in this study was a clay loamy soil with organic carbon 0.91%, total N 0.12%, C: N ratio 8.3, total P 0.072% and CaCO₃ 3.4% and Na concentration was traces and negligible. The soil was air-dried then sieved through 2-mm sieve before using.

Pots were kept in a net house under natural conditions where temperature range was $32\pm 9^{\circ}\text{C}$ maximum and $17\pm 5^{\circ}\text{C}$ minimum. All pots were irrigated with tap water for 7 days, and seedlings were thinned after emergence to four seedlings per pot. At 8th day, pots were irrigated with 0.0, 100 and 200 mM NaCl. After two weeks of applying salinity stress, maize plants were harvested and subjected to measurements of growth parameters. Fresh and dry samples of leaves were kept for biochemical analysis.

HPLC analysis

Both of phenolic and flavonoids were estimated by using HPLC-MS system (Agilent 1100). It is composed of a quaternary pump, a photodiode-array detector, a UV/Vis detector, and a single quadrupole MS detector with ion source (ESI).

Phenolics were separated, according to the method of [58], by employing a gradient mobile phase (within 60 min) of water/acetonitrile/glacial acetic acid (980/20/5, v/v/v, pH 2.68) and acetonitrile/glacial acetic acid (1000/5, v/v) with flow rate at 3 mL/min and detection at 325 nm. On the other side, flavonoids were estimated by using the method of [51]. For separation, a gradient solvent system of 0.1% formic acid solution (within 70 min) with flow rate at 1.0 mL/min, detection at 280 nm and identification by ESI-MS.

Biochemical analysis

Proline was estimated in fresh leaf samples by ninhydrin application according to methods of [10], while GB was estimated by using potassium tri-iodide solution according to method of [23]. Free amino acids were determined following the ninhydrin method [26], and soluble protein was investigated by folin reagent as described by [38]. Total lipids were measured by the method of [12], while total phenols were measured by using Folin-Ciocalteu reagent [3].

To estimate minerals content, dried samples were digested using sulphuric and perchloric acids according to [11]. Content of total K and Na was estimated by flame photometer. Total N was determined using Automatic micro Kjeldahl (Vapodest 30S) according to [3]. Total P and Ca were determined in the digested extracts by Inductively Coupled Plasma Spectrometry (ICP) (Ultima 2 JY Plasma), according to the procedure of [18].

Statistical analysis

Two-way analysis of variance (ANOVA) was performed by applying SPSS v 16. The significant differences among means were determined by Duncan's multiple range test at $P=0.05$. Values were expressed as means of replicates (at least $n=3$) \pm SE.

RESULTS AND DISCUSSION

Phenolic compounds are a class of

the most important and common plant allelochemicals in the ecosystem [13]. Gomaa and Abdelgawad (2013) [22] studied the allelopathic effect of *E. colona* and attributed this effect to its phenolic contents. HPLC analysis (Table 1 and Fig. 1a) revealed that *E. colona* leaves has 8-phenolic acids which acting as allelochemicals; coumaric acid, gallic acid, salicylic acid, syringic acid, ferulic acid, caffeic acid, cinnamic acid and chlorogenic acid (in a descending order). Compounds belong to flavonoids (Table 1 and Figure 1b), a large group of plant secondary metabolites that acting as strong antioxidants and play an important role in plant stress tolerance [14, 17], also were detected.

Salinity impacts growth negatively owing to its osmotic and ion toxicity effects, in addition to the consequent oxidative stress. It was reported that maize growth is stunted and exhibited severe wilting at 250 mM NaCl [40]. In the current study both salinity and EAAE affected significantly maize growth in terms of shoot and root lengths, while salinity×EAAE interaction was significant only for shoot length (Table 2). In response to salt stress, maize significantly declined its shoot and root lengths and the decline was concentration dependent (Table 3). Inhibition of maize growth in response to salt stress is well reviewed and documented

[19], nonetheless this inhibitory effect depends on the genotype and growth interval. Also, negative impact of salinity is known to be more observed on maize shoot than root [19]. In the current study, although hydropriming, shoot length and root length declined to 51.7% and 64.3% respectively of those grown in absence of salinity. Reduced leaf growth rate comes as a rapid response to salinity-induced osmotic stress, while other late symptoms as suppressed leaf initiation, decreased internode growth and accelerated abscission are due to salinity-induced toxicity [19]. Applying *E. colona* allelopathic aqueous extract (EAAE) at 1% stimulated maize growth criteria and significantly reduced the decline that was exhibited by salt stress in shoot and root lengths (Table 2). At severe salinity (200 mM NaCl), shoot length of maize developed from seeds primed with 1% EAAE increased to 148.6% of that belongs to hydroprimed seeds, indicating the alleviation of the adverse effect imposed by salinity on maize growth. Similar growth enhancement under salt stress was reported after priming of wheat seeds with sorghum extract [7].

Chemical priming, hormonal priming, osmo-priming and even hydropriming; all were proved to enhance growth of many plants particularly under stress conditions [4]. Seed priming was

found to help plants to withstand salt stress, but at 3% EAAE maize growth was inhibited in absence as well as presence of salinity when compared with that of the hydroprimed. Analysis of *E. colona* extract revealed the presence of caffeic acid, ferulic acid, cinnamic acid, salicylic acid and coumaric acid.

These allelochemicals revealed an enhancement of salt and osmotic tolerances by enhancing osmo-adjustment and ionic balance and activating antioxidant enzymes when exogenously applied to plants at low concentrations [49, 54, 36, 15]. In contrast, the high concentrations induced negative allelopathic effect [36, 29]. The potentiality of plant extract as an enhancing priming agent was reported to be related with concentration [56, 20].

In response to salt stress, plants adopt some physiological strategies including developing their osmoregulation mechanisms and antioxidative defenses. Salinity and seed priming with EAAE main effects as well as their interactions were significant for all the physiological criteria investigated in the current study (Table 2). Salinity stress led to an accumulation of proline and GB significantly in the tested maize leaves (Table 3). Amplified accumulation of these osmolytes is a common response to salinity in many plants such as tomato, kidney bean, pea, rice, wheat, barely, and maize [25, 53, 21, 50].

The dose 1% EAAE augmented the accumulation of proline and GB under the stressful and non-stressful conditions (Table 3), as compared with control (hydroprimed in absence of salinity). Although proline and GB accumulation was listened at 3% in the unstressed leaves, it amplified to the highest levels at the salt-stressed ones. Proline and GB are two compatible solutes working as osmolytes and osmoprotectants. Their accumulation in plant cell is associated with the enhanced tolerance of the plant to salt and osmotic stress. They accumulate in cell to raise its osmotic potential against the external lower water potential caused by salinity [50]. Thus, they help in the osmoregulation and sustaining cell turgidity. Both also have a role in stabilizing membranes and maintaining the quaternary structures of enzymes and complex proteins under stress conditions [2]. Besides, they have a role in signaling and scavenging ROS generated by stress [34]. Despite the protective role of these osmoprotectants, their highest accumulation in the leaves treated with 3% EAAE in combination with NaCl might come on the account of growth.

Additionally, the highest accumulation of these solutes is likely to be due to cell injury by two combined stresses; salt stress and stress due to the high concentration of allelochemicals, and not to be an essential factor alleviating negative results of stress

[8].

The obtained accumulation of free amino acids due to salinity stress (**Table 3**) may be attributed to its osmotic activity as a mechanism for raising of plant osmotic potential [25]. Another explanation was obtained by [45] who attributed such accumulation to proteolysis and decline of protein synthesis. This attribution is congruent with our data (**Table 3**) showing deterioration in soluble protein content due to salinity. Similar results were obtained by [41] in *Beta vulgaris* and [44] in *Portulaca oleraceae*. The higher increment of amino acids and the lower loss of soluble protein contents in leaves treated with 1% EAAE relative to those in hydroprimed ones, propping the effectiveness of EAAE in preserving macromolecules including soluble proteins and consequently enhancing anabolism and growth against the adverse effects of salt stress. In the same regard, priming with sorghum and garlic extracts increased the content of soluble proteins in wheat under salt stress [7] and *Vicia faba* under drought stress [32], respectively. Similar protective role was shown when regarding total lipids (**Table 3**) that significantly lessened by salt stress, while showed higher levels in samples treated with EAAE combined with salinity. This protective role could be achieved via scavenging the ROS that provoked by stress and upset typical

metabolism via oxidative damage of proteins, nucleic acids and lipids [57], securing the osmoprotectants that work as a shelter for these macromolecules and/or protecting the macromolecules anabolizing enzymes as well as inhibiting the catabolizing ones [5]. All these, on the contrary, were not executed by the higher concentration of extract (3%) that seemed to be exerting more suffering on the salt-stressed leaves, leading finally that maize leaves showed its lowest values of total soluble proteins and total lipids. Inhibition of lipids in response to salt stress was also observed in broad bean [24] and sorghum [43].

Phenolic compounds are secondary metabolites, which participate in plant resistance against stress factors. In this investigation, both salinity and EAAE significantly enhanced total phenolics accumulation (**Table 3**). The accumulation of phenolic compounds due to salinity stress and brimming with plant extracts is recognized in previous studies [1, 56, 17, and 47]. Such accumulation might arise as one of the defense mechanisms employing the antioxidative activities of phenolic compounds, particularly that concerns extinguishing strongly oxidative free radicals such as the hydroxyl radical [47].

While salinity significantly augmented Na^+ accumulation, priming with EAAE effectively declined that content in

the stressed leaves (**Table 3**). Although both concentrations (1 and 3%) kept a higher $K^+ : Na^+$ ratio, the concentration 1% EAAE was more effective in enhancing that ratio particularly at lower salt stress levels. Maintenance of higher K: Na ratio was found to be linked with salt tolerance, as the disruption of protein production comes as a result of raised concentrations of Na^+ that competes with K^+ which is essential for the binding of tRNA to ribosome [52]. Our results support those reported by [7], who found that leaves developed from wheat seeds primed with sorghum water extract had less Na^+ and more K^+ contents than those developed from hydropriming in response to salinity. Similarly, priming pea seeds with *Typha angustifolia* leaves aqueous extract reduced Na^+ and increased K^+ and P amounts in pea aerial parts below those developed from not primed seeds under salt stress [21]. In the current study, the higher $K^+ : Na^+$ ratio due to EAAE priming seems to be correlated with Na^+ exclusion or uptake restriction rather than selectivity of K^+ over Na^+ and the preferential loading of K^+ rather than Na^+ into the xylem [52]. Among the documented toxic effects of Na^+ to maize is the interference with uptake and translocation of other essential mineral elements such as potassium, calcium, nitrogen and phosphorus, leading to decreasing their levels in maize leaves [19].

Calcium level was dimensioned by both salinity and EAAE priming. Nevertheless, EAAE primed tissues displayed a higher Ca^{2+} content than the hydroprimed ones under severe salinity.

In the same context, melatonin as a seed priming agent increased total phenolic content, K^+ , Ca^{2+} as well as $K^+ : Na^+$ and $Ca^{2+} : Na^+$ ratios in the leaves of faba bean (*Vicia faba* L.) under salt stress [16]. In spite of maintaining higher $K^+ : Na^+$ and $Ca^{2+} : Na^+$ ratios due to priming with 3% EAAE over the hydropriming and even priming with of 1% EAAE, leaves developed from 3% EAAE priming showed a dramatic drop of N and P contents under both salt stressful and non-stressful conditions (**Table 3**), referring that the negative effect of 3% EAAE could be more linked to its inhibitory effect on anabolism rather than on membrane integrity and ion balance. In contrast, 1% EAAE significantly induced higher N and P content as compared with the hydropriming under salt stress, proposing a mitigation of the harmful effect of salinity via enhancing membrane integrity and mineral nutrition. Application of lower allelochemical doses to improve plant growth was studied by many researchers like [28] who used lower dose of eucalyptus to improve maize growth and [27] who used lower dose of olive waste to ameliorate the bean plant grown under salt stress.

The positive effect of EAAE priming could be due to the direct role of its phenolic constituents in regulation of ion translocation and mineral balance. It was reported that priming wheat and rice seeds with apigenin and coumarin resulted in higher $K^+ : Na^+$ ratio under salt stress [49, 39]. Reduced Na^+ accumulation in rice leaves was found to be associated with Na^+ exclusion and restriction of uptake via root membranes and the higher expression of SOS1 responsible for Na^+ extrusion and the repression of CNGC1 responsible for Na^+ uptake [39]. EAAE phenolic constituents to scavenge ROS resulted from salt stress [34]. Ismail *et al.*, (2016) [31] applied exogenous rutin to glycophyte bean leaves which improved tissue tolerance and reduced detrimental effects of salinity on leaf photochemistry. These beneficial effects were attributed to improved potassium retention and increased rate of Na^+ -pumping from the cell. The lack of

correlation between rutin-induced changes in K^+ and H^+ fluxes suggests that rutin accumulation in the cytosol scavenges hydroxyl radical formed in response to salinity treatment thus preventing K^+ -leak via one of ROS-activated K^+ -efflux pathways, rather than controlling K^+ -flux via voltage-gated K^+ -permeable channels.

Finally, it could be concluded that EAAE at lower dose (1%) effectively alleviated the harmful effect of salinity on maize growth and physiological processes. The enhancing role may be attributed to its potential to protect macromolecules as soluble proteins and lipids. In addition, EAAE enhanced ionic homeostasis and nutrients uptake by increasing $K^+ : Na^+$ and $Ca^{2+} : Na^+$ ratios and amplifying P and N contents in maize leaves under salt stress.

Table 1: Phenolic acids and flavonoids constituents of *Echinochloa colona* leaves

Phenolic compound	Retention time	Concentration ($\mu g g^{-1} DW$)
Coumaric acid	41.19±0.297	4.1
Gallic acid	35.93±0.261	1.9
Salicylic acid	34.83±0.254	6.9
Syringic acid	27.53±0.204	7.4
Ferulic acid	27.31±0.202	5
Caffeic acid	26.18±0.194	3.4
Cinnamic acid	20.14±0.153	5.5
Chlorogenic acid	10.65±0.088	2.9
Rutin	2.71±0.034	3.5
Quercetin	2.47±0.032	4.1
Catechin	1.91±0.029	1.6
Apigenin	1.21±0.024	5.7
Kaempferol	1.16±0.023	2.9
Hesperetin	0.65±0.020	4.9

Table 2: Mean squares and probability for maize morphological criteria as affected with *Echinochloa colona* allelopathic aqueous extract (EAAE), salinity and their interactions.

Source	df	ShL	RL	Prol	GB	AA	Protein	TL
EAAE	2	1284***	885***	5915***	5918***	6062***	485***	6006***
Salinity	2	1123***	232***	26342***	26355***	26979***	422***	10069***
EAAE×Salinity	4	24.2***	14.7 ^{NS}	9502***	9507***	9741***	19***	2896***

	TPh	Na ⁺	K ⁺	K ⁺ : Na ⁺	Ca ²⁺	N	P	
EAAE	2	5918***	0.34***	25.36***	1464***	0.83***	0.78***	0.094***
Salinity	2	26352***	1.42***	22.1***	5218***	0.72***	0.68***	0.083***
EAAE × Salinity	4	9506***	0.193***	0.984***	1137***	0.03***	0.03***	0.004***

HP; hydroprimed, EAAE; *Echinochloa* allelopathic aqueous extract, Prol; Proline, GB; glycine betaine, AA; amino acids, TL; total lipids, TPh; total phenols, NS Nonsignificant, *** Significant at P≤ 0.001

(a)

(b)

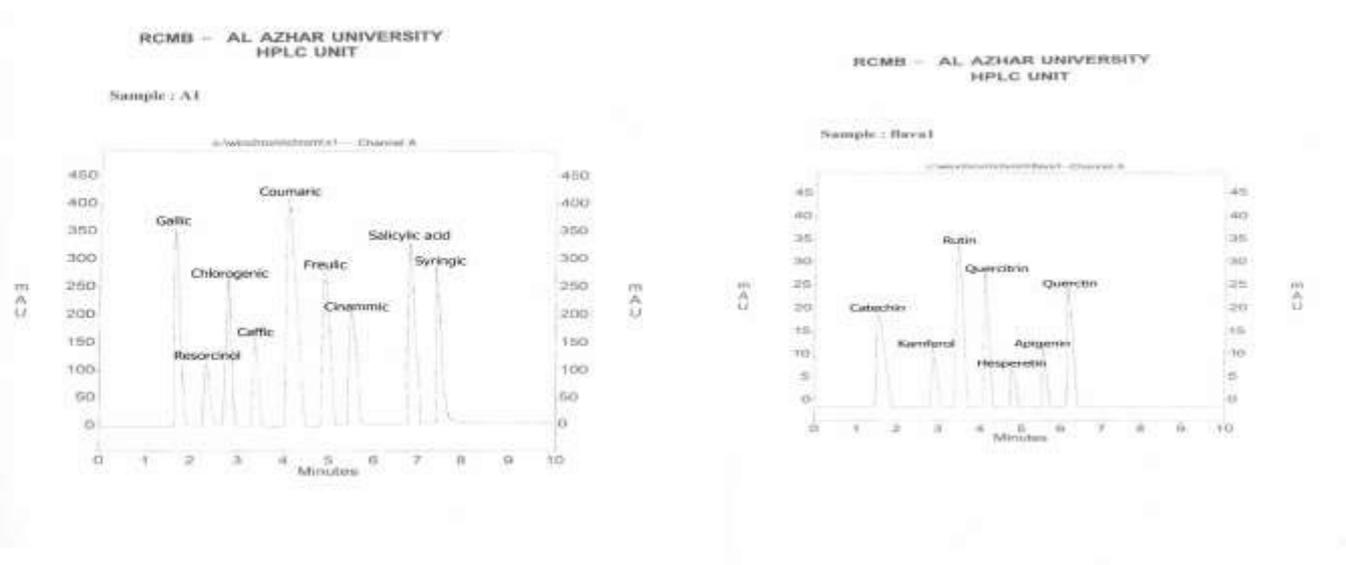


Figure 1: HPLC chromatogram detecting (a) phenolic acids and (b) flavonoids in dried *E. colona* leaves

Table 3: Effect of *Echinochloa colona* allelopathic aqueous extract (1 and 3%) on growth and physiological criteria of maize in presence and absence of salt stress (100 and 200 mM NaCl)

mM NaCl	HP			1% EAAE			3% EAAE		
	0	100	200	0	100	200	0	100	200
ShL (cm)	56±1.0 ^h	34.6±0.58 ^d	29.6±1.5 ^c	61.6±2.9 ^l	50.3±1.5 ^g	44±1.0 ^f	39.6± 1.53 ^c	26.1±1.3 ^b	18.5±1.3 ^a
RL (cm)	39.3±2.5 ^B	28.7±2.5	25.4±1.3	43.3±1.5 ^C	38.6±1.2	37.6±0.9	25.3±3.8 ^A	19.4±0.67	15.5±3.9
Prol (µg g ⁻¹ DW)	231.7±0.48 ^b	259.3±0.71 ^c	270.4±0.94 ^e	264.8±0.67 ^d	278.8±0.49 ^f	295.7±0.62 ^h	187.7±0.6 ^a	326.9±0.41 ⁱ	367.01±1.6 ^j
GB (µg g ⁻¹ DW)	322.3±0.67 ^b	360.8±1.0 ^c	376.1±1.3 ^e	368.4±1.0 ^d	387.8±0.7 ^f	411.3±0.9 ^h	261.1±0.8 ^a	454.7±0.6 ⁱ	510.5±2.2 ⁱ
AA (mg g ⁻¹ DW)	17.6±0.04 ^b	19.7±0.05 ^b	20.6±0.07 ^e	20.1±0.05 ^d	21.2±0.03 ^f	22.5±0.05 ^h	14.3±0.04 ^a	24.8±0.03 ⁱ	27.9±0.12 ^j
Protein (%)	6.41±0.35 ^h	3.54±0.06 ^d	2.78±0.04 ^e	6.67±0.20 ^h	5.61±0.08 ^g	4.73±0.11 ^f	4.15±0.06 ^e	2.27±0.32 ^b	1.56±0.29 ^a
TL (%)	2.76±0.002 ⁱ	1.09±0.01 ^d	0.97±0.003 ^c	1.75±0.04 ^h	1.48±0.01 ^g	1.37±0.01 ^f	1.22±0.01 ^e	0.84±0.003 ^b	0.71±0.01 ^a
TPh (mg g ⁻¹ DW)	1.30±0.003 ^a	1.46±0.004 ^b	1.52±0.00 ^d	1.59±0.003 ^f	1.57±0.002 ^c	1.67±0.003 ^g	1.50±0.003 ^c	1.84±0.002 ^h	2.06±0.008 ⁱ
Na ⁺ (%)	0.42±0.006 ^d	0.55±0.012 ^e	1.23±0.01 ^h	0.26±0.01 ^b	0.11±0.02 ^a	0.93±0.01 ^g	0.37±0.012 ^c	0.36±0.006 ^c	0.83±0.0 ^f
K ⁺ (%)	6.93±0.39 ^{cd}	7.02±0.06 ^d	6.93±0.04 ^d	5.84±0.22 ^a	7.11±0.09 ^e	6.47±0.13 ^{bc}	6.29±0.07 ^b	6.11±0.36 ^b	6.75±0.33 ^c
K ⁺ :Na ⁺	16.5±0.51 ^d	12.8±0.05 ^c	5.6±0.04 ^a	22.5±0.40 ^e	64.6±0.01 ^f	7.0±0.08 ^b	17.0±0.13 ^d	17.0±0.36 ^d	8.1±0.29 ^b
Ca ²⁺ (%)	1.25±0.07 ^h	0.78±0.012 ^f	0.56±0.01 ^c	0.69±0.04 ^e	0.47±0.02 ^b	0.64±0.03 ^d	0.4±0.01 ^a	0.65±0.06 ^d	0.82±0.05 ^{fg}
N (%)	1.26±0.07 ⁱ	0.70±0.012 ^d	0.55±0.006 ^c	1.31±0.04 ⁱ	1.10±0.02 ^h	0.93±0.02 ^f	0.81±0.012 ^c	0.45±0.06 ^b	0.31±0.057 ^a
P (%)	0.44±0.026 ⁱ	0.24±0.006 ^d	0.190±0.0 ^e	0.46±0.015 ⁱ	0.39±0.006 ^h	0.32±0.006 ^f	0.29±0.006 ^e	0.16±0.021 ^b	0.11±0.020 ^a

HP; hydroprimed, EAAE; Echinochloa allelopathic aqueous extract, Prol; Proline, GB; glycine betaine, AA; amino acids, TL; total lipids, TPh; total phenols. Different alphabetical letters indicate significant values at P= 0.05. Small letters are for multiple comparison, while capital letters are for main effects of EAAE when nonsignificant interaction

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