Pertanika J. Trop. Agri. Sci. 47 (3): 721 - 732 (2024)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Evaluation of Foliar Application of *Elusine indica* Extract on Growth, Photosynthesis, and Osmoprotectant Contents in Maize under Drought Stress

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ABSTRACT

To investigate the effects of foliar application of different concentrations of *Elusine indica* extract (EIE) on growth, photosynthesis, and osmoprotectant contents in maize under drought stress. The weed powder was extracted using methanol, followed by a solid-liquid extraction procedure. Plants were sprayed with three different concentrations of EIE at 1, 3, and 5 g/L and morphological parameters, chlorophyll, relative water content (RWC), soluble sugar, proline, protein, glutathione (GSH), and malondialdehyde (MDA) contents were determined. The results showed that drought stress led to a decline in morphological characteristics, RWC and soluble sugar and increased proline, protein, GSH, and MDA contents. However, foliar application of EIE significantly improved plant height, fresh and dry weight, chlorophyll content, RWC, soluble sugar, and GSH, while the proline level was diminished compared to drought treatment. Soluble sugar showed a significant positive correlation with fresh and dry weight (r = 0.742 and 0.783, $p < 0.01$) and a strong negative correlation with MDA (r = -0.459, $p < 0.05$). Therefore, this result indicated that EIE can be used as an inexpensive and environmentally friendly biostimulant to help plants enhance tolerance to drought.

Keywords: Biostimulant, drought tolerance, maize, plant physiology, weed extract

ARTICLE INFO

Article history:
Received: 25 September 2023
Accepted: 18 January 2024
Published: 08 August 2024

DOI: https://doi.org/10.47836/pjtas.47.3.09

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ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Plants depend on their root systems to absorb water from the soil for sustaining growth, while drought stress, as a major abiotic stress factor, severely affects the growth and development of crops and reduces the yield (Gupta et al., 2020; Mohammadi Alagoz, Zahra, et al., 2023). With the exacerbation of the greenhouse effect, drought presents multi-region characteristics, is multi-frequency and unpredictable, causes economic losses, and threatens global food security (Trenberth et al., 2014). The growing population further exacerbates the challenge of meeting food demands in agriculture. Agricultural drought mainly depends on the evapotranspiration of plants, and only sufficient soil moisture can meet the water needs of plants (Goñi et al., 2018). Consequently, in arid regions, irrigation is the most direct and effective strategy to address crop water deficits. However, using irrigation can also give rise to issues such as high costs and soil salinisation. Additionally, the improper application of fertilisers and pesticides can further exacerbate the situation.

Drought induces plant apparatus such as chloroplasts and mitochondria to produce excessive reactive oxygen species (ROS), and the elevated ROS levels subsequently result in physiological disorders, reduced stomatal conductance, and degraded pigment, which negatively affect growth and development (Batra et al., 2014). Besides, studies have shown that drought stress increased the accumulation of proline, soluble sugars, and electrolyte leakage as well as decreased growth indices, relative water content, and triticale yield (Mohammadi Alagoz, Hadi, et al., 2023). Plants have developed intrinsic regulatory mechanisms, such as regulation of stomatal closure antioxidant enzyme activity and overexpression of genes, to improve drought stress tolerance, but these endogenous regulations are often inadequate (Hossain et al., 2017). Biostimulants have received widespread attention due to their positive effects in promoting plant growth, improving nutrient uptake efficiency and plant stress resistance (Goñi et al., 2018; Pourghasemian et al., 2020; Taha et al., 2020). Biostimulants are defined as "materials that contain substance(s) and/or microorganisms, whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independent of its nutrient content" (European Biostimulants Industry Council [EBIC], n.d.). Studies have shown that cypress leaf extract improves photosynthesis and antioxidative defence in zucchini seedlings under salt stress (ElSayed et al., 2022), and Wang et al. (2022) reported that pig blood-derived protein hydrolysate alleviates drought stress in tomato plants.

Maize (*Zea mays* L.) is a major economically important crop cultivated extensively worldwide. The maize yield is particularly sensitive to water scarcity during critical growth stages, such as the tasselling stage, because water deficit leads to a decline in pollen vigour, which further causes the reduction of corn kernels and ultimately causes yield losses (Song & Jin, 2020). Thus, water deficit is the predominant factor limiting maize yield worldwide (Shemi et al., 2021). Studies have been reported to evaluate the effects of biostimulants on maize growth. Chen et al. (2023) reported that applying glycine betaine improves yield and water use efficiency (WUE) in maize under water deficit. Moreover, Tadros et al. (2019) illustrated that treated sweet corn using PERFECTOS® exhibited positive effects on agronomic performance.

Elusine indica is a common annual weed widely distributed in Asia, Africa, and South America and is extremely difficult to control because of its extreme stress resistance (Adoho et al., 2021). In addition, it is also considered one of the five most harmful invasive weeds, which usually grow in fields and compete with crops for nutrients, seriously affecting crop growth and yield (Okokon et al., 2010). Previous studies have illustrated that *E. indica* has anti-inflammatory, antimicrobial, and antioxidant activity (Iqbal & Gnanaraj, 2012). Based on the above content, the scientific and effective utilisation of weed resources can alleviate the detrimental impact of weeds on crops. Furthermore, extracts from weeds may serve as an economical biostimulant to promote plant growth. Thus, the hypothesis of this study is that EIE possesses antioxidant activity and has the potential to enhance drought tolerance, thereby stabilising plant growth. However, no study has been carried out to determine the effects of EIE on mitigating the negative impacts of drought stress on maize. To the best of our knowledge, the objectives of this study were to (1) quantify chemical constituents of EIE, (2) evaluate the effect of foliar spraying EIE on the growth, chlorophyll, and osmoprotectant contents of maize under drought stress, and (3) determine the reasonable concentration for foliar application of EIE. The findings of this study will provide a theoretical basis for the efficient utilisation of weed resources and the production of cheap biostimulants.

MATERIALS AND METHODS

Preparation and Analysis of EIE

All the chemicals were purchased from Chemiz (Malaysia). The *E. indica* weed was collected from a local crop field. The materials were extracted using the detailed method of Taha et al. (2020) with modifications. Each 10 g of materials was mixed with 80% methanol using an optimum ratio of 1/8 (w/v) sample weight to solvent volume. The mixtures were continuously shaken at 150 rpm for 24 hr via an orbital shaker. The extraction samples were filtered with Whatman No.1 filter paper (Cytiva, United Kingdom), and the alcohol and excess water were evaporated under vacuum at 30°C using a rotary evaporator (CCA-111, EYELA, Japan). The EIE obtained from 10 g materials was made as an extract at a concentration of 1.0 g/L by dissolving in 10 L distilled water. The extracts were kept in the refrigerator at 4°C until use.

The EIE was analysed, and its chemical constituents (on a dry weight basis) are shown in Table 1.

Table 1
Chemical constituents of Elusine indica *extract (on a dry weight basis)*

Component	Unit	*Elusine indica* extract
Total carbon (C)	%	46.10
Total nitrogen (N)	%	3.69
Total phenolic	mg GAE/g	13.51
Total flavonoid	mg Lutin/g	26.66
Free proline	mg/g	9.97
Soluble sugars	mg/g	13.23
Protein	mg/g	19.15
Glutathione (GSH)	mg/g	24.01
Phosphorus (P)	g/kg	0.32
Potassium (K)	g/kg	62.08
Calcium (Ca)	g/kg	0.39
Magnesium (Mg)	g/kg	1.31
Iron (Fe)	mg/kg	52.00
Copper (Cu)	mg/kg	4.29
Zinc (Zn)	mg/kg	61.82
Manganese (Mn)	mg/kg	6.72
Boron (B)	mg/kg	3.20

Plant Material and Growing Conditions

This experiment was conducted from March to June 2023 in a glasshouse located at the Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia, with coordinates approximately 2° 98' N and 101° 73' E. Maize seeds (*Zea mays* L., hybrid F1 316, Malaysia) were procured from a local supplier (Agroniche Sdn. Bhd., Malaysia). Four seeds were sown in plastic pots with a diameter of 24 cm and a depth of 28 cm. Each pot was filled with sandy soil totalling 14 kg; the ratio of sand and soil is 1 to 4. The basic properties of soil are total carbon 1.55 g/kg, total nitrogen 2.10 g/kg, total phosphorus 3.87 g/kg, available phosphorus 0.53 g/kg, total potassium 3.40 g/kg, and CEC 4.51 cmol/kg. A completely randomised design (CRD) was designed, and an equal amount of water was added to each pot until drought stress was imposed. After two weeks, the healthiest seedlings were retained, while the others were removed. A total of 15 g compound fertiliser (N: P: K, 15:15:15) was applied to each pot: 5 g at sowing, 5 g after 28 days, and 5 g after 56 days, respectively. The average net greenhouse temperature during the day was 38.80±2.60°C, while during the night, it was 27.52±1.39°C. Additionally, the average net relative humidity during the day was 40.14±4.45%; at night, it was 82.00±4.42%.

EIE Treatments and Experimental Design

The plants grew under normal water conditions until tasselling (56 days). Then they were divided into five treatments: CK (well-watered, 75% soil field capacity), drought (35% soil field capacity), EIE1 (drought with 1 g/L EIE), EIE3 (drought with 3 g/L EIE), and EIE5 (drought with 5 g/L EIE). The concentrations of 1 and 5 g/L were selected based on previous studies by Pourghasemian et al. (2020) and Taha et al. (2020), and an intermediate concentration of 3 g/L was also designed. Each treatment was performed in 4 repetitions. EIE was applied by foliar spraying once a week, totalling four applications. Care was taken to ensure that each application covered all the leaves evenly.

A soil moisture meter monitored each pot's moisture daily, and appropriate volumes of water were added to maintain the desired soil water conditions. After the final spray (84 days), the top leaves of maize were collected and stored at -80°C until they were ready to analyse plant physiology and biochemistry.

Growth Characteristics and RWC

Plant height was measured using a rule. The plant's fresh weight (FW) was measured immediately, and the dry weight was measured after the sample was placed in the oven to constant weight.

Leaf RWC was assessed using the methods described by Wang et al. (2022). Briefly, the leaves were measured to FW and immersed in distilled water for 24 hr to record the turgid weight (TW). Subsequently, the leaves were subjected to oven drying to determine their dry weight (DW). The RWC was calculated using the following formula:

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

Determination of Chlorophyll Content

The contents of chlorophylls and carotenoids were assessed and then calculated, as detailed in Pourghasemian (2020). Samples of 0.3 g fresh squash leaves were extracted in 20 ml 80% acetone and left in the dark. After 24 hr of incubation, absorbance (A) was recorded at wavelengths of 646.8, 663.2, and 470 nm using an Absorbance Microplate Reader (Tecan Trading AG, Switzerland).

Determination of Osmoprotectant

Soluble sugar content was determined using the anthrone colourimetric method (Dubois et al., 1951). Briefly, 0.3 g fresh maize samples were homogenised with 10 ml 80% ethanol and then centrifuged at 10,000 × *g* at 4°C for 10 min using a centrifuge (D-78532, Hettich, Germany). The resulting extract was mixed with 5ml anthrone reagent and then heated at 100°C in a water bath for 10 min, and the absorbance was read at 630 nm after cooling to room temperature. Proline quantification was determined following the method of Bates et al. (1973), and the absorbance was read at 520 nm. The protein content was measured according to Bradford (1976) using bovine serum albumin as the standard.

Determination of GSH and Lipid Peroxidation

GSH contents were quantified using the method described by Griffith (1980). Briefly, 0.3 g fresh leaves were extracted with 10 ml 5% trichloroacetic acid (TCA) and centrifuged at 4,000 × *g* for 15 min. The 2 ml of extract was mixed with 0.4 ml of 5, 5'-dithiobis (2-nitrobenzoate) (DTNB); the absorbance was read at 412 nm.

MDA indicated lipid peroxidation following De Vos et al. (1991). The 2 ml of extract (the same as GSH) was mixed with 2 ml of 0.6% 2-thiobarbituric acid (TBA). The mixture was heated at 95°C for 15 min and cooled immediately in an ice bath. The absorbance of the supernatant was read at 532 nm after centrifugation at 4,000 × *g* for 10 min.

Statistical Analysis

Data were analysed using the SPSS (ver. 25.0). Significant differences between treatments were calculated by one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test at $p < 0.05$.

RESULTS

Morphological Characteristics

Table 2 indicates that fresh and dry weight and number of leaves were ($P < 0.01$) influenced significantly by drought and EIE treatments. Compared to the CK treatment, drought stress decreased plant height, fresh and dry weight, and number of leaves by 3.6, 42.2, 48.5, and 13.6%, respectively. In addition, the application of EIE exhibited a positive effect on all assessed morphological characteristics under drought conditions. Among them, EIE treatments increased plant height by 2.9–15.1%, fresh weight by 51.6–64.6%, dry weight by 11.8–71.2%, and number of leaves by 7.1–11.9%.

Table 2
Effect of drought stress and foliar application on growth of maize

Treatment	Plant height (cm)	Fresh weight (g)	Dry weight (g)	No. of leaves
CK	165.15±11.62ab	165.18±7.51a	64.47±4.49a	12.15±0.50a
Drought	159.15±10.34b	95.53±7.58c	33.23±2.31b	10.50±0.58b
EIE1	183.13±14.76a	144.79±10.67b	37.67±2.81b	11.25±0.50b
EIE3	163.87±14.84ab	147.29±9.47b	53.03±2.62ab	11.75±0.50ab
EIE5	171.70±11.80ab	157.28±11.80ab	56.88±7.36ab	11.50±0.58ab
Source of variation				
Drought	-0.3	-0.983**	-0.981**	-0.882**
EIE	0.452	0.937**	0.916**	0.655**

Note. Different lowercase letters indicate significant differences between the treatments ($p < 0.05$); Analysis of variance results of the main effects and their interaction effect on the growth of maize are shown as ** as significantly as $p < 0.01$, respectively; CK = Well-watered; EIE1 = Drought with 1 g/L EIE; EIE3 = Drought with 3 g/L EIE; EIE5 = Drought with 5 g/L EIE; EIE = *Elusine indica* extract

Chlorophyll Content and RWC

Table 3 illustrates that drought and application of EIE affected chlorophyll content and RWC responses of maize. Drought treatment increased chlorophyll *a*, chlorophyll *b*, and carotenoid content by 48.5, 3.5, and 13.1%, respectively, and decreased RWC by 11.6%, indicating that less water resulted in a high chlorophyll content. EIE1 treatment exhibited the highest content of chlorophyll *a*, chlorophyll *b*, and carotenoid with increases of 57.4 and 5.9%; 36.5 and 31.8%; and 44.1 and 27.3% compared to the CK and drought treatments. Besides, applying EHE improved RWC under drought conditions, in which the EHE5 treatment exhibited the highest RWC and was significantly higher than all other treatments ($p < 0.05$).

Table 3
Effect of drought stress and foliar application on chlorophyll and relative water content (RWC) of maize

Treatment	Chl *a* (mg/g)	Chl *b* (mg/g)	Carotenoid (mg/g)	RWC (%)
CK	2.04±0.11c	2.55±0.15b	2.36±0.21c	60.08±5.63b
Drought	3.03±0.24ab	2.64±0.13b	2.67±0.14bc	53.12±4.69b
EIE1	3.21±0.19a	3.48±0.35a	3.40±0.34a	58.17±9.14b
EIE3	2.85±0.26b	2.62±0.06b	2.78±0.28b	57.92±2.90b
EIE5	3.02±0.20ab	2.50±0.10b	2.65±0.08bc	71.20±6.67a
Source of variation				
Drought	0.95**	0.35	0.713*	-0.613
EIE	-0.014	0.231	0.323	0.466

Note. Different lowercase letters indicate significant differences between the treatments ($p < 0.05$); Analysis of variance results of the main effects and their interaction effect on the chlorophyll (Chl) and RWC are shown as * and ** as significantly as $p < 0.05$ and 0.01, respectively; CK = Well-watered; EIE1 = Drought with 1 g/L EIE; EIE3 = Drought with 3 g/L EIE; EIE5 = Drought with 5 g/L EIE; EIE = *Elusine indica* extract

Osmoprotectants, GSH, and MDA

Table 4 shows that drought and application of EIE had significant main effects on proline, sugar, protein, GSH, and MDA ($p < 0.05$ and 0.01). Drought significantly increased proline, protein, GSH, and MDA content by 50.6, 13.7, 308.3, and 1.78%, respectively, and decreased sugar by 31.4% compared to CK treatment ($p < 0.05$). In addition, application of EIE improved sugar by 15.7–20.7%, protein by 6.3–11.1%, GSH by 18.4–26.5%, and MDA by 18.5–14.4%, and decreased proline by 17.8–20.3% compared to drought treatment.

Correlation and Principal Component Analysis

The results presented in Table 5 indicated several noteworthy correlations among the examined characteristics. Notably, a significant positive correlation was observed between sugar content and fresh weight ($r = 0.742$), dry weight ($r = 0.783$), and number of leaves ($r = 0.754$) ($p < 0.01$).

Conversely, sugar content exhibited a strong negative correlation with proline (*r* = -0.754), MDA (*r* = -0.459), and GSH (*r* = -0.68). Furthermore, the data revealed a positive correlation between GSH and proline (*r* = 0.523) as well as MDA (*r* = 0.768). Additionally, proline exhibited a significant negative correlation with fresh weight (*r* = -0.726), dry weight (*r* = -0.751), and the number of leaves (*r* = -0.675) ($p < 0.05$ and 0.01).

Table 4
Effect of drought stress and foliar application on osmoprotectants, glutathione (GSH), and malondialdehyde (MDA) of maize

Treatment	Proline (μg/g)	Sugar (mg/g)	Protein (mg/g)	MDA (μmol/g)	GSH (mg/g)
CK	136.84±19.35c	2.04±0.09a	1.83±0.13b	13.19±0.60c	0.12±0.03c
Drought	206.12±13.95a	1.40±0.14c	2.08±0.11a	14.97±0.94b	0.49±0.07b
EIE1	164.24±15.98b	1.69±0.13b	2.21±0.16a	15.96±1.03ab	0.58±0.06ab
EIE3	169.40±17.11b	1.67±0.10b	2.26±0.14a	16.89±1.38b	0.59±0.13ab
EIE5	168.40±10.01b	1.62±0.09b	2.31±0.18a	17.12±1.51a	0.62±0.08a
Source of variation					
Drought	0.921**	-0.954**	0.848**	0.792*	0.969**
EIE	-0.797**	0.738**	0.268	0.535*	0.49*

Note. Different lowercase letters indicate significant differences between the treatments ($p < 0.05$); Analysis of variance results of the main effects and their interaction effect on the proline, sugar, MDA, and GSH are shown as * and ** as significantly as $p < 0.05$ and 0.01, respectively; CK = Well-watered; EIE1 = Drought with 1 g/L EIE; EIE3 = Drought with 3 g/L EIE; EIE5 = Drought with 5 g/L EIE; EIE = *Elusine indica* extract

Table 5
Correlation coefficients of total morphological, chlorophyll, and osmoprotectant characteristics

	PH	FW	DW	NL	Chl *a*	Chl *b*	Caro	RWC	Proline	Sugar	Protein	MDA	GSH
PH	1												
FW	0.31	1											
DW	0.159	0.971**	1										
NL	0.035	0.776**	0.814**	1									
Chl *a*	0.126	-0.54*	-0.657**	-0.613**	1								
Chl *b*	0.399	-0.038	-0.127	-0.235	0.446*	1							
Cart	0.262	-0.108	-0.226	-0.329	0.688**	0.865**	1						
RWC	0.25	0.234	0.229	0.02	0.097	-0.12	-0.105	1					
Pro	-0.18	-0.726*	-0.751**	-0.675**	0.446*	0.002	0.121	-0.305	1				
Sugar	0.133	0.742**	0.783**	0.754**	-0.689**	-0.032	-0.194	0.015	-0.754**	1			
Protein	0.256	-0.287	-0.425	-0.229	0.589**	0.096	0.266	-0.002	0.340	-0.445*	1		
MDA	-0.061	-0.011	-0.118	-0.212	0.566*	0.069	0.293	0.22	0.269	-0.459*	0.539*	1	
GSH	0.201	-0.294	-0.415	-0.491*	0.75**	0.182	0.409	0.131	0.523*	-0.68**	0.737**	0.768**	1

Note. PH = Plant height; FW = Fresh weight; DW = Dry weight; NL = Number of leaves; Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*; Caro = Carotenoid; RWC = Relative water content; MDA = Malondialdehyde; GSH = Glutathione; * and ** as significantly as $p < 0.05$ and 0.01, respectively

DISCUSSION

Water deficit reduces the plant's capacity to uptake carbon dioxide and decreases stomatal conduction, affecting photosynthesis, and ultimately limiting plant growth. In this study, foliar application of EIE can significantly mitigate negative effects caused by water deficit by regulating photosynthesis, osmotic adjustment, and antioxidant defence. Interestingly, drought stress decreased plant height, fresh and dry weight, and number of leaves of maize plants, whereas application of EIE exhibited positive effects on plant growth (Table 2). This result is consistent with Anjum et al. (2011), who reported that drought stress reduced the gas exchange, limiting maize growth and productivity. The improved effect in this study can be attributed to the fact that EIE is abundant in essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as osmoprotectants like soluble sugars and proline, and antioxidant including phenolic compound and flavonoids (Table 1).

The presence of these crucial components in EIE gives it the potential to mitigate the adverse effects of drought stress. Moreover, Taha et al. (2020) reported that foliar spraying of pollen grain extract could enhance the growth characteristics of basil and pointed out that biostimulant alleviates drought stress by promoting cell division and elongation and repairing plant nutritional status. Additionally, our finding demonstrated that applying EIE improved the chlorophyll contents and leaf RWC of stressed maize plants, reflecting the enhanced photosynthetic capacity and water uptake efficiency. This finding further confirms the beneficial effects of EIE in enhancing drought tolerance.

Plants' exposure to drought will stimulate the regulation of their internal physiological and biochemical metabolic systems and reduce oxidative damage by enhancing drought tolerance (Rai et al., 2012). One protective mechanism is that accumulating osmoprotectants such as proline and soluble sugar helps plants regulate stomatal pressure and enhance photosynthesis (Gill & Tuteja, 2010). A previous study found that pumpkin seed protein hydrolysate could improve the tolerance of *Phaseolus vulgaris* to salt stress by increasing the content of essential nutrients, proline, and soluble sugar (Sitohy et al., 2020). Moreover, Taha et al. (2020) reported that pollen grains extract increased proline and soluble sugar content in *Ocimum basilicum*. In the present study, EIE treatments led to a significant increase in soluble sugar content while causing a decline in proline content under drought stress conditions (Table 4). This result is similar to those found by Pourghasemian et al. (2020), who thought that under drought stress, foliar application with biostimulants led to a substantial effect on inducing plants' drought tolerance by improving protein and antioxidant content as well as reducing proline levels, further enhancing gas exchange and chlorophyll content.

GSH is a major low molecular-weight antioxidant compound in plants, and it acts as a redox-active molecule and plays a crucial role in regulating the ascorbic acid - glutathione (AsA-GSH) cycle (Galant et al., 2011). This study showed that applying EIE significantly increased GSH content compared to drought treatment (Table 4). This finding was consistent with Taha et al. (2020), who reported that GSH and AsA can alleviate the damage from excessive accumulation of ROS, allowing plants to establish an internal defence mechanism to resist drought-induced oxidative stress. MDA production can be used as an indicator to evaluate the oxidative stress-induced free radical damage of cell membranes (ElSayed et al., 2022). Wang et al. (2022) reported an increase in MDA concentration in tomato plants under drought stress, which was markedly reduced after pig blood-derived protein hydrolysate application. Similarly, Pourghasemian et al. (2020) reported that foliar application of liquorice extracts reduced MDA content in sesame and mitigated drought stress.

However, our study showed that the application of EIE increased the level of MDA and had a positive correlation with the increase in concentration. This may be due to improper use of phytostimulant concentrations or the presence of phytohormone-stimulated oxidation in EIE itself. In addition, the findings from Goñi et al. (2018) illustrated that after adding seaweed extract, the MDA concentration in plants exhibited an initial decrease over a specific period, followed by an increase towards the end of the experiment. This also indicated that the effect of biostimulants is time-sensitive, and the application frequency is critical for substantial effects. Overall, EIE as a biostimulant is more beneficial to plants than harmful. The rational utilisation of weed resources can effectively reduce the direct competition with crops for nutrients and provide a feasible formula for a biostimulant application strategy.

CONCLUSION

Foliar application of EIE significantly improves growth performance and photosynthesis capacity by increasing chlorophyll and leaf RWC and accumulating soluble sugar and GSH. This finding indicates that EIE has the potential as a mitigator to mitigate adverse effects caused by drought stress. Moreover, *E. indica* is easy to obtain. Thus, EIE can be used as an inexpensive biostimulant to enhance plant tolerance through foliar spraying. However, the effects of EIE application on maize yield and intrinsic gene regulation are unclear and will be verified in future field trials.

ACKNOWLEDGEMENTS

This paper was supported by Universiti Putra Malaysia, Innohub Grant Scheme (Vote no. 9005004), and D' Khairan Farm Sdn. Bhd., Selangor, Malaysia (Vote no. 6300349).

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