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Antioxidant properties and biostimulant potential of an Iraqi *Prosopis farcta* fruit extract in basil under contrasting soil-moisture regimes

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Abstract
Background: *Prosopis farcta* belongs to the family of Fabaceae and is of ethnomedicinal value but its agricultural potential is not sufficiently explored.
Objectives: To assessed antioxidant capacity and biostimulant activity of a *P. farcta* fruit extract on basil (*Ocimum basilicum* L.) grown under different soil-moisture levels was tested.
Methodology: A 4×2 factorial experiment with five replications was conducted using the pot method and varying the concentration of extracts (0, 0.5, 1.0 and 2.0 g L–1) in combination with soil-moisture levels (100% and 60% field capacity).
Results: Dry-extract yield was 18.6 ± 0.8%. The total phenolic and flavonoid contents were of 82.4 ± 2.7 mg GAE g^{-1} and of 36.8 ± 1.9 mg QE g^{-1} dry extract, respectively. DPPH radical-scavenging activity reached 71.3 ± 2.1% at 1.0 g L^{-1}, with an IC_{50} of 0.42 ± 0.03 g L^{-1}. Extract concentration and irrigation main effects were significant with regard to growth, water status pigment status, oxidative stress and soluble protein as determined by two-way ANOVA ($p<0.001$). None of the interactions between treatments was significant for growth, SPAD, relative water content or soluble protein; except H_2O_2 ($F_{3,32} = 7.34$, $p<0.001$). At 60% FC, biomass and the amount of soluble protein was descriptively greater, while H_2O_2 was descriptively lower, than the untreated control, and post hoc comparisons on those data were not examined.
Conclusion: This extract demonstrated some initial promise as a botanical biostimulant however, replication, field testing, toxicity and food-safety evaluation are needed before further recommendations.

Keywords: *Prosopis farcta*, *Ocimum basilicum*, biostimulant, hydrogen peroxide, oxidative stress

Introduction

Plant species that survive under water-limited conditions, high temperature, high radiation and unpredictable rainfall are present in dry and semi-arid regions. *Prosopis farcta* (Banks & Sol.) (*P. farcta*) J.F. Macbr. is a perennial plant of the family Fabaceae which is broadly distributed in dry and semidried areas and native in Iraq and surrounding areas? Traditionally the plant is used for medicinal purposes, but its traditional use cannot be regarded as evidence for efficacy or safety. It therefore needs to be assessed for agricultural application by an appropriate extraction protocol, chemical characterization and experimental testing in crop plants [1]. The available chemical evidence suggests that *P. farcta* comprises a number of groups of possibly active constituents. The seeds have been reported to be rich sources of protein, unsaturated fatty acids and phenolic material [2]. It cannot be assumed that the use of a fruit extract prepared with aqueous ethanol that reproduces the composition of a leaf, seed or water extract. To be able to interpret any physiological response in the treated plants and to be able to repeat the experiment it is crucial to describe the plant part used and the extraction conditions. The presence of compounds containing measurable reducing and the radical-scavenging properties in the genus *Prosopis* has been documented in previous studies. Polyphenol-enriched fractions have been found to have significant *in vitro* antioxidant activity and various biological activities in reviews of the genus [3]. In addition, an extract of *Prosopis* cineraria with high phenolic content has also been described to be antioxidant and anti-inflammatory in an experimental wound model [4]. It is well established, however, that malondialdehyde (MDA) levels are increased by ethanol exposure, and that *P. farcta* fruit extract, administered in water, prevented this increase [5]. Laboratory antioxidant assays depict reactions in simplified chemical systems, but responses in an intact plant will also be affected by uptake, dose, tissue and environmental factors and interactions between the contents of the extract.

This evaluation can be considered within the framework of the concept of plant biostimulant. A biostimulant is used to enhance nutrient-use efficiency, abiotic stress tolerance, or crop-quality traits, with or without providing a significant quantity of mineral nutrients [6]. A reproducible improvement in plant performance (yield or other agronomically relevant criterion) at an agronomically relevant dose can meet this definition in the case of botanical extracts. Their effects could be due to the cumulative effect of numerous components and not just the single compound. This pattern is characteristic of mixtures ving mixtures and is consistent with the general idea that responses to biostimulants reflect a number of interactions with plant metabolism, signaling, and the environment of the rhizosphere and/or the phyllosphere [7]. Both positive and negative effects can occur, and a concentration series is needed to determine what range of concentrations is beneficial, not necessarily what results in the highest production. Water shortage affects plant development as it inhibits cell expansion, reduces carbon fixation and a affect cellular hydration, and disturbs redox balance. In normal metabolism, free radicals are constantly generated and at certain levels may be involved in transmitting cellular messages. Loss of cellular control may lead to the

accumulation of hydrogen peroxide and other oxidants, which can damage pigments, modify proteins and lead to lipid peroxidation [8]. Normally the amount of malondialdehyde-equivalent TBA substances (TBARS) approximates lipid oxidation and relative water content (RWC) and chlorophyll-related measurements (chlorophyll a, b and a/b) give complementary information concerning leaf hydration as well as physiological status. The effect of these constituents, phenolic and flavonoids compounds, may be at the chemical redox level or related to growth processes [9].

Basil (*Ocimum basilicum* L.) is well suited for evaluating this because it is a economically important culinary, aromatic and medicinal crop species that is sensitive to water. On the other hand, research on the basil landraces has revealed that the irrigation level affects dry-matter and essential oil yield and that there are differences between landraces in the ability to yield under conditions of limited irrigation water [10]. Basil is also sensitive to treatments with biostimulant materials of biological origin: applications with cyanobacterial preparations in controlled conditions have resulted in an increase in growth, leaf number and fresh plant weight [11]. The results indicate that basil is sensitive to novel inputs, with the type of response it causes and the extent of the response being a function of how the input was prepared, the concentration of the input, the cultivar being used, and the growing environment. The properties of locally sourced materials will consequently have to be determined rather than assumed in relation to an commercial products with different compositions. Although the interest in *P. farcta* is chemical, and in the agriculture sector the need for safe and cost-effective biostimulants has grown in water-limited regions, there is not enough direct evidence about the effect of locally collected *P. farcta* fruit extract on Iraqi basil. This gap was addressed in the present study, through the evaluation of the extraction yield, total phenolics, flavonoids and *in vitro* antioxidant activity of the extracts at four different concentrations prepared from the fruits and also in low and high soil moisture conditions. Plants height, leaf number, leaf area, biomass, SPAD, RWC, hydrogen peroxide and soluble protein were measured. This study amid to assessed antioxidant capacity and biostimulant activity of a *P. farcta* fruit extract on basil (*Ocimum basilicum* L.) grown under different soil-moisture levels was tested.

Methodology

Experimental design

The investigation was conducted by such a balanced factorial design that was balanced 4 × 2 factorial pot experiment. Factor A consisted of four levels: four different concentrations of the extract of *P. farcta* (0, 0.5, 1.0 and 2.0 g dry-extract equivalent L^{-1}). Factor B was two soil moistures, 100% FC and 60% FC. Thus, there were 8 combinations of treatments: Pots served as the experimental units with each combination consisting 5 pots; 1 basil plant per pot (total 40 pots). The technical assay readings within pots were not regarded as biological replicates, but as one average per pot.

Plant material and extract preparation

In August 2024, fruits were collected from an orchard in Al-Musayyib District of Babylon Governorate, Iraq. Fruits of *P. farcta* were collected in August 2024 from an orchard located in Al-Musayyib District of Babylon Governorate, Iraq. Morphological identification and confirmation of the material by a plant taxonomist from College of Science, University of Babylon were provided. Fruits were washed with distilled water, dried in the dark at 40 °C to a constant weight and powdered by using a uniform sieve and packed in airtight opaque containers at 4 °C for extraction. The dried fruit powder (100g) was extracted with 10 times its volume of 70% aqueous ethanol for 24 hours with intermittent shaking. The extract was filtered and taken to dryness in vacuo at a temperature below 40 °C, and then dried gently at 25 °C until a constant weight was attained.

Extraction yield (%) = (mass of dry extract / mass of original dry powder) × 100

The dry extract was dissolved in distilled water to prepare 0, 0.5, 1.0 and 2.0 g L^{-1} solutions. The solutions were then filtered through 0.45-µm and used within 24 h. No surfactant or carrier was added to the extract solution, which was dissolved in distilled water. All treatments had the same amount (volume) of application and spraying method, while the control received distilled water only. Three foliar applications were made at seven-day intervals.

Chemical characterization and *in vitro* antioxidant activity

The Folin-Ciocalteu reaction was used to measure the total phenolic content, which was calculated as milligrams of gallic acid equivalent per gram of dry extract (mg GAE g^{-1}). The number of total flavonoids was estimated by aluminium-chloride method and expressed as mg of quercetin equivalent per g of dry extract (mg QE g^{-1}). The radical-scavenging activity was quantified by DPPH assay. Reagent blanks, extract-colour blanks and calibration standards were included. The percentage inhibition (%) of DPPH assay was calculated as follows [12]:

Inhibition (%) = [(A control – A sample) / A control] × 100

Only concentrations which established both sides of 50% response were used to report the IC_{50} (concentrations bracketing the 50% response). DPPH concentration–response curves were generated using extract concentrations of 0, 0.10, 0.20, 0.30, 0.40, 0.50, 0.75, and 1.00 g L^{-1}. Three replicate extract preparation runs were considered as independent analytical replicates. The IC_{50} was calculated as the concentration at which 50% of the DPPH was inhibited and was determined from the concentration – response curve. Extraction yield, TPC, TFC and DPPH activity were determined from three prepared batches of extraction independently. Each batch was repeated three times for three technical measurements, the averages of which were used to obtain one value for the average of each independent batch. Data are presented as mean (plus or minus) standard error (SE) of the three independent batches (n = 3).

Basil production and irrigation control

Uniform seeds of the basil herb were purchased from a local nursery in Babylon Governorate in Iraq and were later transplanted into 5 litres pots filled with a thoroughly homogenized growth medium of agricultural soil, sand and peat moss in ratio of 2,1,1 (v/v). The field capacity of the medium was gravimetrically determined by taking a sample of medium and filling it with water, draining it for 24 h and then weighing and drying the sample in the oven at 105 °C until a constant weight was reached. Field capacity water retention amounts were determined by the difference

between the dry and oven dry masses. The amount of water retained at field capacity for each pot was added to the combined mass of the empty pot and the oven-dry medium to get the target mass at 100% field capacity. The water deficit for the 60% field-capacity treatment was derived from the equation:

$M_{60} = M_{(dry\text{-}pot)} + 0.60 \times (M_{100} - M_{(dry\text{-}pot)})$.

Following medium equilibrium at the moisture level assigned, the pots were weighed each day, and lost water replaced to bring the pot to the moisture level assigned. The 100% and 60% field-capacity levels were maintained until the final harvest. One limitation of the study was that the fertilizer was not described in sufficient detail to determine the formulation and application rate. Environmental, nutritional and cultural conditions were not monitored and controlled between treatments. The cultivar was not determined but the plants were identified as local basil.

After transplantation, 40 pots were divided between eight treatments and there would be five individual pots per treatment and one plant per pot as the experimental unit. Randomisation and the positions of the pots in the greenhouse was not recorded and so may have influenced the results.

Growth, pigment, and water-status measurements

Measuring at final harvest included plant height, the number of leaves and leaf area. Roots and shoots were separated, fresh mass measured and tissues dried to constant mass at 70 °C for dry weight determination. The chlorophyll was measured with the same method in all treatments and expressed as chlorophyll value (SPAD units). The fresh weight (FW), turgid weight (TW) and dry weight (DW) were used to determine the relative water content (RWC):

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

To get the TW value, over the course of 4 h at 25 °C, leaves were rehydrated in distilled water, surface water was carefully shaken off, and leaf weight was immediately determined.

Oxidative-stress indicators

Immediately prior to harvest, young leaves, from the same canopy position, were harvested and stored in liquid nitrogen at −80 °C. The amount of H_2O_2 was measured according to the potassium iodide method of Velikova *et al*., where 0.5 g of leaf frozen in powder form was homogenized on ice in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and then centrifuged at 10,000 × g for 10 min at 4 °C [13]. An aliquot (0.5 mL) of the tissue extract (0.1% TCA) was mixed with potassium-phosphate buffer (pH 7.0) (0.5 mL) and 1.0 M KI (1.0mL) freshly prepared. Absorbance was determined after 60 min in the dark at room temperature by using 1 cm O.D. cuvette. Reagent and sample-color blanks were provided. A series of standards containing H_2O_2 were prepared in 0.1 % TCA and treated in exactly the same way as the samples. From the calibration curve, H_2O_2 concentration was derived and subsequently, it was expressed as µmol g^{-1} fresh mass of tissue (FW) and 5.0 mL of original extraction volume.

Soluble protein determination

Soluble protein was extracted separately from 0.2 g of leaf tissue in 2 mL of cold phosphate buffer and polyvinylpyrrolidone was added, if necessary, to reduce phenol interference. The sample was then homogenized on ice and subjected to centrifugation at 10 × g 4 °C for 10 min. A known amount of the clarified supernatant was then mixed with Bradford reagent and the absorbance read at a standardised incubation time at 595 nm. A bovine-serum-albumin calibration curve was used to determine the protein concentration, expressed as milligrams per gram fresh weight [14]. Reagent blanks and extract-color controls were present and technical readings for each biological sample were averaged prior to statistical analysis.

Statistical analysis

Fixed-effects two-way ANOVA was conducted for analysis of the data produced with extract concentration, irrigation regime and their interaction as fixed effects and pot as the experimental unit. Residual- versus-fitted and Q–Q plots and testing for homogeneity of variance were used to evaluate model assumptions. The tests reported on the ANOVA terms were considered as omnibus tests; no individual comparisons of treatments and controls were made. SPSS version 26.0 was used for analyses at $\alpha = 0.05$.

Results

Extraction yield and *in vitro* antioxidant activity

Dry-extract yield was 18.6 ± 0.8% of the initial amount of fruit powder. The total phenolic and flavonoid contents were 82.4 ± 2.7 mg GAE g^{-1} and 36.8 ± 1.9 mg QE g^{-1} of dry extract, respectively. At 1.0 g L^{-1}, the extract produced 71.3 ± 2.1% DPPH inhibition, and the estimated IC_{50} was 0.42 ± 0.03 g L^{-1} (n=3) (Table 1). These chemical assays are intended to assess *in vitro* radical-scavenging capacity only, and do not on their own confirm antioxidant protection in whole plants.

Table 1: Chemical properties and antioxidant activity of the *P. farcta* fruit extract

Indicator	Mean ± SE	Interpretation
Extraction yield	18.6 ± 0.8%	Dry extract relative to starting powder
Total phenolics	82.4 ± 2.7 mg GAE g^{-1} dry extract	Folin–Ciocalteu reaction
Total flavonoids	36.8 ± 1.9 mg QE g^{-1} dry extract	Aluminum-chloride reaction
DPPH inhibition at 1.0 g L^{-1}	71.3 ± 2.1%	Color-corrected inhibition
DPPH IC_{50}	0.42 ± 0.03 g L^{-1}	Estimated from the concentration–response curve

Growth, biomass, chlorophyll status, and water relations

Extract concentration and irrigation level each affected every growth variable (all $p < 0.001$), whereas their interactions were not significant: plant height, $F_{3,32} = 0.54$, $p = 0.660$; leaf number, $F_{3,32} = 0.18$, $p = 0.907$; leaf area, $F_{3,32} = 0.09$, $p = 0.968$; fresh weight, $F_{3,32} = 0.24$, $p = 0.864$; and shoot dry weight, $F_{3,32} = 0.16$, $p = 0.924$. Irrigation reductions resulted in reduced growth at all concentrations (Table 2). Under 60% FC, the mean values were 34.5% higher than the corresponding untreated-control means for leaf number, 33.6% for leaf area, 40.9% for FW and 43.9% for shoot DW while the mean value of plant height was

35.3% higher. These percentage differences were not analysed using pairwise post-hoc tests. These growth responses were not statistically significant drought-specific mitigation, due to the less significance of the interaction terms.

Table 2: Growth traits under *P. farcta* extract and irrigation treatments

Extract (g L^{-1})	Field capacity	Plant height (cm)	Leaf number	Leaf area (cm^2)
0.0	100%	24.8 ± 0.7	18.4 ± 0.5	322 ± 11
0.0	60%	18.7 ± 0.6	14.2 ± 0.4	238 ± 9
0.5	100%	27.1 ± 0.8	20.1 ± 0.6	356 ± 12
0.5	60%	22.4 ± 0.7	16.7 ± 0.5	276 ± 10
1.0	100%	29.6 ± 0.9	22.8 ± 0.7	391 ± 13
1.0	60%	25.3 ± 0.8	19.1 ± 0.6	318 ± 11
2.0	100%	28.4 ± 0.8	21.7 ± 0.6	374 ± 12
2.0	60%	23.1 ± 0.7	17.8 ± 0.5	294 ± 10

Values are presented as mean ± SE (n = 5 independent pots per treatment combination)

There were significant main effects for both extract and irrigation on SPAD ($p < 0.001$) and RWC ($p < 0.001$) and no significant extract irrigation interaction for SPAD ($p = 0.908$) or RWC ($p = 0.470$). At 1.0 g L^{-1}, under 60% field capacity the mean values of SPAD and RWC were 25.6% and 12.7% greater respectively, than the mean for the untreated controls. Pairwise post-hoc tests of these differences were not conducted (Table 3 & Figure 1). The 1.0 g L^{-1} treatment did not have the lowest means on several traits measured, but did have the most favorable most favorable overall descriptive pattern among the measured traits.

Table 3: Biomass, chlorophyll status, and relative water content

Extract (g L^{-1})	Field capacity	Fresh weight (g)	Shoot dry weight (g)	SPAD	RWC (%)
0.0	100%	38.6 ± 1.2	6.42 ± 0.21	39.8 ± 1.1	86.1 ± 1.4
0.0	60%	27.4 ± 1.0	4.51 ± 0.18	31.2 ± 1.0	72.5 ± 1.6
0.5	100%	43.2 ± 1.3	7.18 ± 0.24	42.7 ± 1.2	88.3 ± 1.3
0.5	60%	32.8 ± 1.1	5.36 ± 0.20	34.9 ± 1.1	76.4 ± 1.5
1.0	100%	47.9 ± 1.4	8.11 ± 0.27	46.1 ± 1.3	91.0 ± 1.1
1.0	60%	38.6 ± 1.2	6.49 ± 0.22	39.2 ± 1.2	81.7 ± 1.3
2.0	100%	46.1 ± 1.4	7.76 ± 0.25	44.5 ± 1.2	89.6 ± 1.2
2.0	60%	35.1 ± 1.1	5.91 ± 0.21	36.8 ± 1.1	78.5 ± 1.4

Values are presented as mean ± SE (n = 5 independent pots per treatment combination)

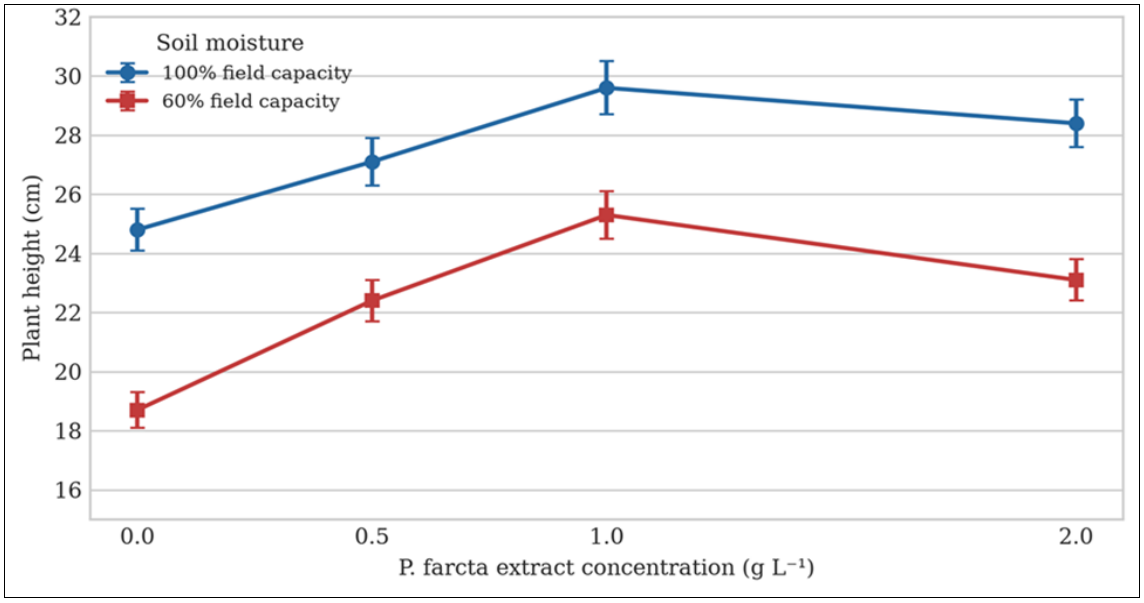

Fig 1: Plant height under four *P. farcta* extract concentrations and two soil-moisture regimes. Points are means and error bars are SE (n = 5 pots).

Oxidative-stress indicators

Both extract concentration and irrigation regime significantly influenced the leaf-H_2O_2 concentration (at $p < 0.001$). There was also a significant extract × irrigation interaction ($F_{3,32} = 7.34$, $p < 0.001$, $\eta p^2 = 0.408$), meaning that the concentration-response relationship was different in each irrigation treatment (Table 4). Descriptively, the H_2O_2 concentrations presented as average for the 1.0 g L^{-1} application at 60% FC were descriptively 37.1% lower than the untreated control. No comparative individual treatment/control analyses, however, were made.

Table 4: Leaf H_2O_2 under extract and irrigation treatments

Extract (g L^{-1})	Field capacity	H_2O_2 (µmol g^{-1} FW)
0.0	100%	4.8 ± 0.2
0.0	60%	8.9 ± 0.3
0.5	100%	4.3 ± 0.2
0.5	60%	7.1 ± 0.3
1.0	100%	3.7 ± 0.2
1.0	60%	5.6 ± 0.2
2.0	100%	3.9 ± 0.2
2.0	60%	6.4 ± 0.3

Values are presented as mean ± SE (n = 5 independent pots per treatment combination)

Soluble protein concentration

Concentrations of soluble-protein were significantly affected by extract concentration ($p < 0.001$) and irrigation level ($p < 0.001$) but not by interaction ($F_{3, 32} = 0.46$, $p = 0.711$, $\eta p^2 = 0.041$) (Table 5). Soluble protein levels were reduced because of reduced irrigation at all extract levels. The mean concentration (mg g^{-1} FW) of soluble-protein increased by 27.0% at 1.0 g. L^{-1} compared to the untreated control under 60% FC. Individual comparisons were not performed by pair wise post hoc test. In the no significant interaction, there was no statistical evidence that the concentration effect would differ between moisture regimes and therefore, there is no evidence that thesetwo concentration–response profiles are equivalent.

Table 5: Soluble-protein concentration under extract and irrigation treatments

Extract (g L^{-1})	Field capacity	Soluble protein (mg g^{-1} FW)
0.0	100%	18.6 ± 0.6
0.0	60%	15.2 ± 0.5
0.5	100%	19.7 ± 0.6
0.5	60%	17.1 ± 0.5
1.0	100%	21.4 ± 0.7
1.0	60%	19.3 ± 0.6
2.0	100%	20.6 ± 0.6
2.0	60%	18.2 ± 0.5

Values are presented as mean ± SE (n = 5 independent pots per treatment combination)

Factorial analysis of variance

Complete factorial ANOVA is found in (Table 6). Most of the outcomes exhibited high magnitude of main effects, particularly those of irrigation on H_2O_2 ($\eta p^2 = 0.895$), fresh weight ($\eta p^2 = 0.822$) and RWC ($\eta p^2 = 0.817$). The effects of the interaction with the treatments were negligible with respect to growth, SPAD, RWC and soluble protein; but they were significant with a relatively large value for the H_2O_2.

Table 6: Two-way ANOVA for variables measured at both irrigation levels

Response	Effect	df	F	p	ηp^2
Plant height (cm)	Extract concentration	3, 32	20.08	<0.001	0.653
Plant height (cm)	Irrigation level	1, 32	91.26	<0.001	0.740
Plant height (cm)	Extract × irrigation	3, 32	0.54	0.660	0.048
Leaf number	Extract concentration	3, 32	25.65	<0.001	0.706
Leaf number	Irrigation level	1, 32	93.16	<0.001	0.744
Leaf number	Extract × irrigation	3, 32	0.18	0.907	0.017
Leaf area (cm^2)	Extract concentration	3, 32	16.31	<0.001	0.605
Leaf area (cm^2)	Irrigation level	1, 32	102.54	<0.001	0.762
Leaf area (cm^2)	Extract × irrigation	3, 32	0.09	0.968	0.008
Fresh weight (g)	Extract concentration	3, 32	25.66	<0.001	0.706
Fresh weight (g)	Irrigation level	1, 32	147.41	<0.001	0.822
Fresh weight (g)	Extract × irrigation	3, 32	0.24	0.864	0.022
Shoot dry weight (g)	Extract concentration	3, 32	24.84	<0.001	0.700
Shoot dry weight (g)	Irrigation level	1, 32	128.96	<0.001	0.801
Shoot dry weight (g)	Extract × irrigation	3, 32	0.16	0.924	0.015
SPAD	Extract concentration	3, 32	13.88	<0.001	0.565
SPAD	Irrigation level	1, 32	90.32	<0.001	0.738
SPAD	Extract × irrigation	3, 32	0.18	0.908	0.017
RWC (%)	Extract concentration	3, 32	9.55	<0.001	0.472
RWC (%)	Irrigation level	1, 32	142.74	<0.001	0.817
RWC (%)	Extract × irrigation	3, 32	0.86	0.470	0.075
H_2O_2 (µmol g^{-1} FW)	Extract concentration	3, 32	30.38	<0.001	0.740
H_2O_2 (µmol g^{-1} FW)	Irrigation level	1, 32	271.68	<0.001	0.895
H_2O_2 (µmol g^{-1} FW)	Extract × irrigation	3, 32	7.34	<0.001	0.408
Soluble protein (mg g^{-1} FW)	Extract concentration	3, 32	12.99	<0.001	0.549
Soluble protein (mg g^{-1} FW)	Irrigation level	1, 32	41.14	<0.001	0.562
Soluble protein (mg g^{-1} FW)	Extract × irrigation	3, 32	0.46	0.711	0.041

Discussion

Folin–Ciocalteu-reactive content was found to be 82.4 ± 2.7 mg GAE g^{-1} dry extract, and Aluminium chloride -reactive flavonoids content was 36.8 ± 1.9 mg QE g^{-1} dry extract, without identification of individual compounds, but with measurable DPPH radical- scavenging activity. Assessments of these have included comparative evaluations and have shown that the assays relate to each other, therefore they can be used as complementary, but not interchangeable, methods for assessing the same thing [15]. A similar chemically diverse phenolic profile is found to be correlated with radical-scavenging activity in another species of *Prosopis* [16]. In a more general context, methodological reviews highlight that antioxidant assays may involve different physicochemical principles, and therefore should not be lumped together in one universal value [17]. The dose response was not necessarily proportional. The 1.0 g L^{-1} concentration showed the most advantageous overall pattern, while the concentration 2.0 g L^{-1}, in general, showed a smaller benefit. An application test with a plant-derived seaweed extract applied to cucumber plants at harvest also revealed dose and application dependent responses with basil, where higher concentrations are not necessarily more effective [18]. Since no pairwise post hoc comparisons, nor a dose-response model were performed, it is not considered to be a statistically determined optimum concentration. The effective concentration might change on another batch or in another environment due to fruit maturity, collection site, drying, solvent conditions and foliar retention.

Plant height, leaf number, leaf area, fresh weight, shoot dry weight, SPAD, and relative water content were significantly reduced with reduced irrigation. Another study conducted in the field for 2 years also showed that deficit irrigation caused a decrease in the growth as well as fresh and dry yield of purple basil [19]. A pot experiment confirmed that severe water deficit was linked to decreases in RWC, increases in MDA, and reductions in biomass of shoots of sweet basil - WBCAOB [20]. Under drought, genotype-dependent relationships between water status, SPAD reading, photosynthesis and biomass also have been reported [21]. The simultaneous enhancement of those endpoints (all) after the application of *P. farcta* therefore represents a more broadly systemic effect and does not mean that the application results in a change in only one of the measurements. The factorial results improve the interpretation of this. Extract concentration x irrigation level interactions were not significant whereas extract concentration and irrigation level had significant main effects on growth and water-status variables. Significant extract concentration (in the absence of an interaction between concentrations and moisture regimes) means that the response was different among extract concentrations averaged over moisture regimes. The omnibus test alone does not, however, tell which concentrations differed from the untreated control. The use of Moringa leaf extract in a defined application condition in a field experiment has also established a botanical preparation to enhance the yield, protein content and soil nutrient uptake [22]. The trend observed is in line with a response from a candidate biostimulant for the various moisture regimes but further repetition in field trials is needed for confirmation.

This is different from the statistics results for hydrogen peroxide. Drought-associated ROS can be regarded as context dependent signals or causes damage when production outstrips the ability to maintain cellular redox control [23]. The quantity of H_2O_2 and MDA is determined and increases over the course of the drought process, and the amount of these compounds is genotype-dependent, so these compounds can be used as complementary indicators of stress responses [24]. In the present study, a descriptively lower concentration of H_2O_2 than the untreated stressed was observed when using 1.0 g L^{-1} with an average difference of 37.1%. The extract × irrigation interaction was significant, thus resulting in higher biochemical responses to H_2O_2 than that found to the growth traits under low-soil-moisture conditions. The decrease in H_2O_2 levels were associated with a lower oxidative load but do not indicate the mechanism nor a decrease in membrane injury. The response observed could have been due to phenolics or flavonoids present in the formulation; it was not possible to determine whether or which of these two constituents were responsible for the observed response, nor could it be determined if they were absorbed through the foliage. This explanation must thus be regarded merely as a hypothesis and cannot be proved as a mechanism [25]. There are indirect possibilities as well, if the extract acted in beneficial ways and produced results in the areas of hydration, pigment retention, membrane stability or metabolic regulation. Independent membrane-damaging markers, and time resolved sampling would help clarify the mechanism of the current design.

The effect of extract concentration and irrigation on soluble protein was significant whereas extract concentration × irrigation was not significant. Effect of water limitation resulted in a decrease in protein concentration whereas, with 1.0 g L^{-1}, there was an increase in the protein concentration by 27.0% compared to the untreated stressed control. The sensitivity of soluble-protein responses varies with stress intensity, genotype, and may not always be consistent in association with drought tolerance [26]. The rise seen here may be indicative of general metabolism stability, maintenance of soluble components of cells or enhanced hydration of tissues, and cannot be attributed to any protein class. Although polyvinylpyrrolidone is added and blank correction is performed to compensate for nonenzymatic reaction, and a calibration range has been decided upon, these remain significant when comparing dye-binding protein measurements between treatments.

Overall, the results in this study offer some preliminary evidence that *P. farcta* fruit extract has potential as a botanical source of botanical biostimulants. Meta-analysis of field trials indicates that biostimulant responses are different depending on the type of product, application technique, crop, climate and soil properties, which is confirmed by standardized field trials [27]. Crops can also respond differently to preparations made from equivalent plants that exhibit chemical variation and it is important that the chemical composition of the botanical can be characterised on a batch-by-batch basis [28]. The 1.0 g L^{-1} response will be further determined for independent extract batches, other basil genotype, under different seasons and through field-scale evaluation. By standardisation through the phenolic profile or using another chemical fingerprint, it will be determined whether or not different collections provide the same performance. The study did not aim at evaluating phytotoxicity, extraction components residues on food tissues, antinutritional factors, microbial effects and

food safety. Therefore, recommendations for using basil as food aren't justified from the present results.

Conclusion

Foliar application of *P. farcta* fruit extract in this one pot experiment was correlated with concentration-dependent variations in the growth, biomass, SPAD, RWC, soluble protein and H_2O_2 content of basil. The observed mean pattern was most favorable in the 1.0 g L^{-1} treatment, although there were no individual post hoc comparisons to assess if this was significantly different from the untreated control or the other concentrations tested. The irrigation × extract interaction was significant for H_2O_2, which means that the concentration-response curve for H_2O_2 was different depending on soil-moisture regime. The results support further research but do not justify recommendations for edible basil. The independent replication, extract standardization, field validation, phytotoxicity assessment and residue and food-safety evaluation are required.

Conflict of Interest

There is no conflict of interest in this article.

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