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Biostimulants combination enhance the metabolites content of Brassica *oleracea* var. *botrytis* curds cultivated in the desert region of Basrah governorate

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Abstract

Background

Among Brassica vegetables, cauliflower (*Brassica oleracea* var. *botrytis*) is widely recognized as a most consumed vegetable due to its rich and healthy

content of nutrients and bioactive compounds. The enhancement of productivity and quality of preferred crops has gained highly importance in recent decades, driven by increasing customers demand for affordable and healthy food. Away from chemical materials and their risky side effects, biostimulants and biofertilizers could be a suitable alternative for traditional fertilizers under desert conditions.

Methodology

In a current study the extract of Liquorice root LRE and bread yeast BYE applied both with 0, 10, and 20 g/L on Brassica plant to explore their influence in curds metabolites. The results demonstrated that while carbohydrates reduced in curds of treated plants, protein increased with both stimulants application. The non-significant increasing in phenols and flavonoids content was observed in curds of LRE and BYE-treated plants with an elevation in antioxidant activity in their methanolic extracts. Both LRE and BYE applications promoted the curds content of macronutrients and caused a notable alteration in active compounds profile. Noteworthy, the values of acquired outcomes increased or decreased according to applied concentrations of individual or combination of both biostimulants.

Conclusions

Therefore, the foliar application with LRE and BYE enhanced the primary and secondary metabolites of Brassica curds. However, the combination of 10 g/L of LRE and 20 g/L of BYE is recommended due to its functions in promoting the flavonoids content, antioxidant capacity, potassium content and active compounds profile such as organosulfur, troponoid, methyl 9-cis,11-trans-octadecadienoate, 1H-Indole-3-acetonitrile and 9,12,15-Octadecatrienoic acid. These findings demonstrate that the synergistic effect of LRE and BYE biostimulants can reprogram primary and secondary metabolism, strengthening stress resilience and improving the nutraceutical quality of cauliflower grown in saline desert environments.

Key words: Biostimulants, Liquorice root extract, Bread yeast extract, Cauliflower curds, Metabolites. Desert region

1- Introduction

To achieve sustainability in the agricultural sector, it is necessary to eliminate synthetic fertilizers and apply instead biostimulants (BIOs) to enhance both the quantity and quality of crops. Biostimulants are substances or/and microorganisms that optimize plant growth through enhance nutrition efficiency, soil fertility, abiotic stress tolerance, and crop quality without direct effect on the nutrients content [1]. BIOs are commercially available in numerous forms and could be designated as product contain mixture of both substances and microorganism. Patrick du Jardin divided biostimulants into seven categories: humic and fulvic acids, protein hydrolysates (PHs), oligopeptides and polypeptides, seaweed and botanical extracts, biopolymers, and beneficial fungi and bacteria [1]. BIOs derived from plants have received a notable attention in the recent years and researchers highly investigated their potential effect on plants growth under normal and stress conditions [2, 3]. Among plants that used their extracts as BIOs, Liquorice (*Glycyrrhiza glabra*) root extract (LRE) is used particularly due to the rich content of osmoprotectants, phytohormones and antioxidants [4]. LRE has been reported as a promising natural biostimulants due to its role in enhancement the plant ability to uptake nutrients [5], promote a good growth and yield [6], and improve abiotic stress tolerance [7, 8].

Numerous active compounds exist in LRE extract such as antioxidants, phenols, flavonoids, triterpenoid and saponins and help to stimulate its favourable role when applied on crops [9]. Likewise, recent utilization of bread yeast *Saccharomyces cerevisiae* extract (BYE) as biostimulants have garnered attention for their potential to enhance plant growth and contribute to sustainable agriculture [10]. BYE derived from yeast cells is rich in effective components such as low-molecular-weight organic substances, amino acids, nucleotides, peptides, nitrogen, phosphorus, and trace elements with absence of chemical hormones and toxic ingredients [11]. The foliar application of BYE on plants can stimulate plant metabolism and enhance the vitality, leading to boost nutrient uptake, increase biomass, improv biochemical traits and consequently optimize the high value productivity in cabbage and tomato crops [12, 13].

White cauliflower Brassica *oleracea* var. *botrytis* is annual plant belongs to the Brassica family, is an edible and highly desirable crop among Iraqi consumers. The edible parts of the plants are immature inflorescences, heads, or curds. Curds are rich with a privileged content of nutrients and active constituents such as fibres, soluble sugars, minerals, carotenoids, vitamins, glucosinolates, polyphenols, and flavonoids [14]. White cauliflower is one of crops that are voracious for fertilizers special the nitrogen. Achieving high and good productivity of crop could be required massive amounts of fertilizers that are costly, polluted, and not ensuring the sustainable requirements. Thus, exploring suitable alternatives such as biofertilizers and biostimulants is necessary to reduce the agriculture cost, optimize the quality and quantity of yield , minimize global carbon footprint and protect our environment [15].

The Basrah governorate is characterized by arid desert conditions with high temperatures, intense solar radiation, and saline soils—factors that collectively impose osmotic and ionic stress on crops. These conditions severely limit plant growth and productivity. Biostimulants such as LRE and BYE have shown potential in mitigating such stress effects. LRE, enriched with proline, betaines, and polyphenols, enhances osmotic adjustment and protects cellular structures against heat and salt-induced oxidative damage [16, 17]. BYE, on the other hand, provides amino acids, vitamins, and phytohormone-like molecules that stimulate antioxidant enzymes and maintain ionic balance [18]. Therefore, the combined application of LRE and BYE may offer synergistic protection against the predominant abiotic stresses of the Basrah desert environment, improving plant resilience and growth performance.

The present study investigates the effects of Lemna root extract (LRE) and Bacillus yeast extract (BYE), applied individually and in combination, as biostimulants on the nutritional value, active metabolite composition, and antioxidant potential of Brassica oleracea var. botrytis (cauliflower) curds cultivated under the desert conditions of Basrah governorate. Specifically, this research evaluates how the combined application of LRE and BYE influences the physiological, biochemical, and metabolomic profiles of cauliflower. By integrating GC-MS-based metabolite profiling with physiological and biochemical analyses, we hypothesize that the biostimulant combination enhances both primary and secondary metabolic pathways, thereby improving stress adaptation, antioxidant defense, and curd quality. The outcomes of this study provide valuable insights into sustainable biostimulant-based strategies for enhancing crop productivity and nutritional quality in environmentally challenging and saline desert agroecosystems.

2- Materials and methods

2.1. Cultivation and treatments

The verity cauliflower hybrid F1 Alnahar seeds from Monarch seeds, Noord-Scharwoude, Holland, cultivated in trays with total peat moss substrate. After

40 days of cultivation, seedlings transferred to permanent field in the southern of Basrah, treatment with biostimulants started after 30 days of transferring. Biostimulants treatments were applied as foliar sprays once every two weeks for a total of three applications throughout the experimental period. Each application was performed in the early morning to ensure optimal absorption. Samples from field soil and irrigation water analysed in the central lab of Soil and Water Resources Department (Table 1) and all experiments conducted in the lab of Medicinal and Aromatic Plants Unit at University of Basrah

` **Table (1): Analysis of the field soil and irrigation water**

Parameter	**Value**	**Unit**
	Soile	
pH	7.7	
ECE	5.22	dS/m
Phosphorus	38.8	mg/kg
Nitrogen	0.23	g/kg
Potassium	101.20	mg/kg
Calcium	16.5	mmos/L
Magnesium	11	
Sodium	21.3	
Carbonates	0.00	
Bicarbonates	13.6	
Sulfates	18.5	
Chlorides	28.0	
Sand	593	g/kg
Silt	271.5	
Clay	135.5	
Soil texture	sandy loam	
	Water	
pH	7.80	
ECE	5.34	dS/m

2.2. Biochemical traits of curds

The cruds of treated plants were dried at room temperature and used to estimate the content of carbohydrates, protein, phenols, flavonoids, and elements as well as their active constituent’s profile

2.2.1. Carbohydrates content

Carbohydrates content was determined by Phenol-Sulphuric Acid method according to [19]. From dried samples 0.5 g homogenized with 70 ml of distilled water at 70 °C for 1 hr. Then, 5 ml of filtrated extract placed in a volumetric flask and made up to 30 ml by distilled water. From diluted extract, 1 ml placed in a new tube and homogenized with 1m of 5% phenol and 5 ml of pure sulfuric acid by vortex. After brining the mixture to the room temperature, the absorption was recorded by spectrophotometer at 490 nm and carbohydrates concentration was measured from glucose standard curve and expressed as mg of glucose equivalents per gram of sample dry weight (mg GU/g dw).

2.2.2. Nitrogen content

Protein content was determined in dried plant samples following Kjeldahl method. From dried samples 0.20 g was digested with 5 ml of pure sulfuric acid at 350 °C for 30 minutes. After the samples cooling, 3 ml of the mixture of 96 ml sulfuric acid and 6 ml perchloric acid was added to the sample solution and placed on digestion plate at 350 °C until the solution being clear. The volume of sample solution was made up to 50 ml by distilled water. From diluted sample 10 ml was mixed with 10 ml of 6 N of sodium hydroxide in the tube and placed in Kjeldahl analyzer instrument. The distillate was collected in a conical flask containing 2 % of Boric acid and 2% of bromocresol green and methyl red indicator solution (0.099 g + 0.066 g in 100 ml Ethanol). The collection was titrated with 2 % HCl until the solution has a slightly violet color, the percentage of nitrogen was calculated through the equation:

$$\%N = ((V_1 - V_0) \times N \times 14.007 \times 100) / (W \times VS)$$

where V_1 is the volume of acid used for sample titration, V_0 is the volume used for blank titration, N is the normality of the acid, W is the weight of the sample (g), and VS is the volume of the digest aliquot used for titration.

2.2.3. Crude protein content

The percentage of crude protein was calculated following the question:

Crude protein = %N X 6.25 (conversion factor)

2.2.4. Total phenols content

Cruds content of phenols estimated by Folin-Ciocalteu method according to [20]. With 5 ml of 80% methanol, 0.25 g of dry sample was homogenized and kept 48 hrs in dark, 24 hrs at room temperature and 24 hrs at 4 °C. To 0.5 ml of the methanolic extract, 0.5 ml of Folin–Ciocalteu reagent was added and followed with 8 ml of distilled water. After 5min of incubation, 1ml of 20% sodium carbonate (Na2CO3) was added. After 30 mins incubation at ambient temperature in dark, an intense blue color developed, and its absorbance was measured at 760 nm. Gallic acid was used as a standard by preparing stock solution at concentration 1 mg/ml and used for the calibration. The results were expressed as mg of gallic acid equivalents per gram of the dry weight sample (mg GAE/g dw)

2.2.5. Total flavonoids content

Total flavonoids content in cruds of control and treated plants was evaluated following [21]. To 0.5 ml of extract that placed in 10 ml tubes, 0.5 ml of 80% methanol added and followed by the adding of 10 ml of distilled water and 0.3 ml of 5% sodium nitrite (NaNO3). After incubation for 5 mins, 0.3 ml of 10% aluminum chloride (AlCl3) and 2 ml of 1M sodium hydroxide (NaOH) were added. The volume was made up to 10 ml with distilled water and vortexed, the absorbance was recorded spectrophotometry at 510 nm after orange-yellowish color development. Quercetin was used as a standard by preparing stock solution at concentration 1 mg/ml and used for the calibration. The results were expressed as mg of quercetin equivalent per gram of the dry weight sample (mg QE/g dw).

2.2.6. Elements composition

Potassium, phosphors, and sulfur were determined by dry ash method [22]. In porcelain crucibles, 1 gm of cruds samples were placed and incinerated at an initial temperature of 200°C for 1h on a hot plate until the smoke disappears. Then, samples kept in Nabertherm muffle furnace at 556 °C for 5 hrs for total incineration. Next, ashes were dissolved by a mixture of 4 ml of nitric acid (65%) and 10 ml of distilled water on a hot plate. The solution was filtered and made up to 25ml with distilled water and used for elements (P, K, S) analysis by atomic absorption/emission spectroscopy. The calibration curves were generated using the standards of each element, and the results were expressed as mg of element per gram of the dry weight sample.

2.2.7. GC-MS analysis

2.2.7.1. Samples preparation

From dried cruds, 0.250 g homogenized with 5 ml of 80 % methanol, vortexed to achieve a good mixing and incubated in dark at room temperature overnight. Next day, supernatant collected carefully by micropipette and sent for injection in GCMC instrument

2.2.7.2. GCMC conditions

GC-MS analysis was carried out by using an Agilent Technologies, 7890B GC system coupled to an Agilent Technologies 5977A MSD with EI Signal detector, using HP-5ms 5% phenyl, 95% methyl siloxane (30m*250um*0.25). The oven temperature was set at 40 °C hold for 5 mins then raised to 8 °C/min to 300 °C for 20 mins, Helium carrier gas flow rate was 1 ml/min and purge flow of 3 ml/min. The injection mode was pulsed Splitless with injection temperature 290 °C and the injection sample volume was 0.5 µl. The mass spectrometer used Ion Source Temperature 230 °C, with scan speed 1562(N2), and the mass range 44-750 m/z. Compound identification was implemented using mass spectral data and retention indices, in comparison with authentic standards and spectral libraries, including the NIST 2014, 2020 database. GC-MS analysis in this study was conducted using relative quantification, where compound abundance was expressed as a percentage of normalized peak areas. This approach was selected to assess compositional variations in metabolite profiles among treatments rather than to determine absolute concentrations. All samples were processed and injected under identical parameters to minimize variability associated with matrix effects and instrument response.

2.3. Experiment design and statistical analysis

The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replicates for each of the nine treatment combinations. Each replicate comprised five individual plants, arranged randomly within each block to minimize environmental variability. Data were collected from all replicates and subjected to Two-Way ANOVA to assess the main and interaction effects of the treatments.

3- Results

Figure 1 illustrates the effect of biostimulants spray on curds content of carbohydrates and proteins, the spray with LRE and BYE at different concentration caused reduce the carbohydrates content of curds compared to the control plants, the range of values between 19.32 mg GU/g dw and 22.86 mg GU/g dw (Figure 1A). On the other hand, protein proportion increased remarkably in curds of 10 g/L of BYE-treated plants (15.89 %) and plants that sprayed with 20 g/L of LRE and 10 g/L of BYE combination (14.87 %) (Figure 1B)

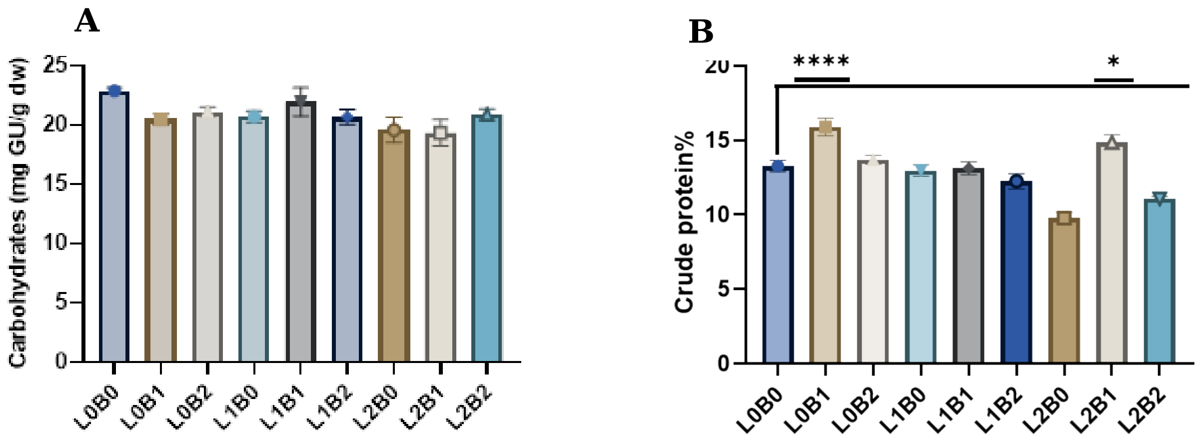


Figure (1): The effect of foliar application with Liquorice root extract (LRE) and Bread yeast extract (BYE) on carbohydrates and crude protein content in Brassica curds. (A) Representative the effect of LRE and BYE on the carbohydrates content of Brassica curds. L0B0 control or untreated plants. L0B1treated plants with BYE at 10 g/L. L0B2 treated plants with BYE at 20 g/L. L1B0 treated plants with 10 g/L of LRE. L1B1treated plants with 10 g/L of LRE and 10 g/L of BYE. L1B2 treated plants with 10 g/L of LRE and 20 g/L of BYE. L2B0 treated plant with 20 g/L of LRE. L2B1 treated plants with 20 g/L of LRE and 10 g/L of BYE. L2B2 treated plants with 20 g/L of LRE and 20 g/L of BYE. (B) Representative the effect of LRE and BYE on the protein content of Brassica curds. A multiple ANOVA was performed using ordinary one-way ANOVA multiple comparisons to compare the averages of treatments with L0B0. Significance was designated as follows: *$p < 0.05$, ***$p < 0.0001$.

Total phenolic content (Figure 2A) and total flavonoids content (Figure 2B) in curds exhibited non-significant increase due to foliar application of both extracts on Brassica plants. The increase of phenols content was in curds of 20 g/L of BYE-treated plants (17.03 mg GAE/g dw), while flavonoids content increased in curds of combination of 10 g/L of LRE and 20 g/L of BYE-treated plant (16.54 mg QE/g dw). Antioxidants activity of curds extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals reinforced in curds methanolic extract of LRE-sprayed plants and BYE-sprayed plants and their combination-sprayed plants recorded 75.00 %, 77.59 %, and 74.09 % respectively (Figure 2C).

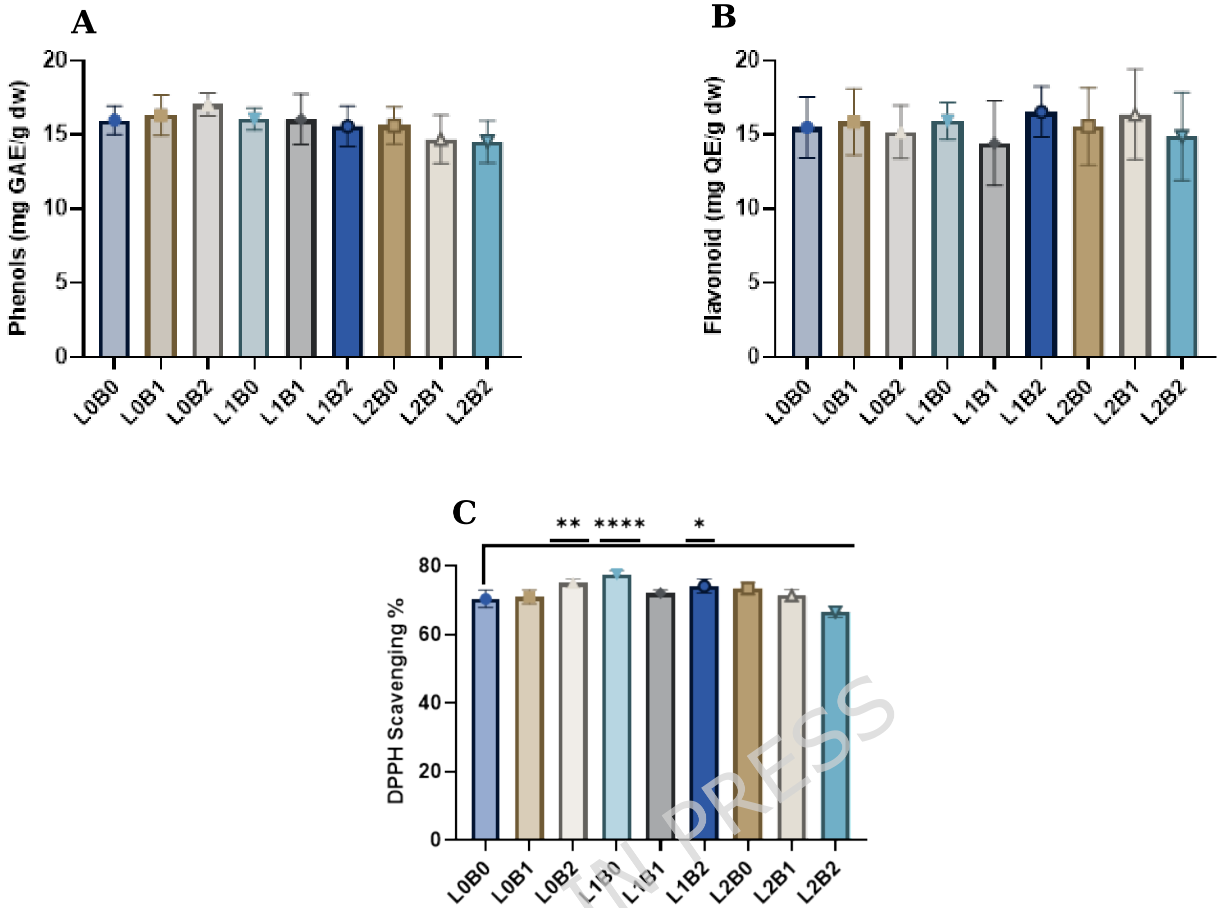
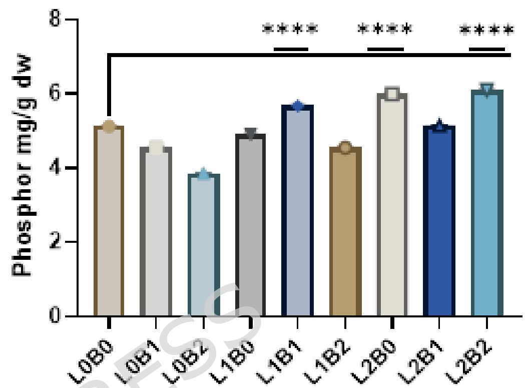


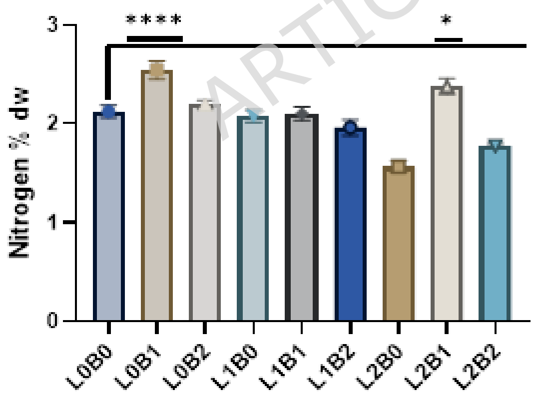
Figure (2): The effect of foliar application with Liquorice root extract (LRE) and Bread yeast extract (BYE) on phenols and flavonoids content and DPPH scavenging in Brassica curds. (A) Representative the effect of LRE and BYE on the phenols content of Brassica curds. L0B0 control or untreated plants. L0B1treated plants with BYE at 10 g/L. L0B2 treated plants with BYE at 20 g/L. L1B0 treated plants with 10 g/L of LRE. L1B1treated plants with 10 g/L of LRE and 10 g/L of BYE. L1B2 treated plants with 10 g/L of LRE and 20 g/L of BYE. L2B0 treated plant with 20 g/L of LRE. L2B1 treated plants with 20 g/L of LRE and 10 g/L of BYE. L2B2 treated plants with 20 g/L of LRE and 20 g/L of BYE. (B) Representative the effect of LRE and BYE on the flavonoids content of Brassica curds. (C) Representative the effect of LRE and BYE on the DPPH scavenging of Brassica curds extract. A multiple ANOVA was performed using ordinary one-way ANOVA multiple comparisons to compare the averages of treatments with L0B0. Significance was designated as follows: *$p < 0.05$, **$P < 0.01$, ****$p < 0.0001$.

Elements composition including nitrogen, phosphors, calcium and sulfur displayed the effect of foliar application of biostimulants on macro elements content in Brassica curds. Nitrogen portion increased in curds of 10 g/L BYE-treated plants and 20 g/L LRE and 10 g/L BYE-treated plants (2.54, 2.38) % (Figure 3A). Phosphor content was impacted with individual and combination applications of LRE and BYE, P content promoted in curds of 10 g/L of LRE and BYE-treated plants (5.67 mg/g), 20 g/L LRE-treated plants (6.00 mg/g) and 20 g/L of LRE and BYE-treated plants (6.10 m/g) of dry weight (Figure 3B). Furthermore, potassium content increased subsequently the foliar spray with l and BYE. Curds of 20 g/L BYE-treated plants (28.83 mg/g), 10 g/L of LRE-treated plants (23.31 mg/g) and the combination 10 g/L of LRE and 20 g/L of BYE-treated plants (28.52 mg/g) of dry weight (Figure 3C). Sulfur content in Brassica curds was boosted in curds of plants that treated with 10 g/L of LRE and the combination of 20 g/L of LRE and 10 g/L of BYE that both recorded 3. 60 mg/g of dry weight (Figure 3D).

A

B





C

D

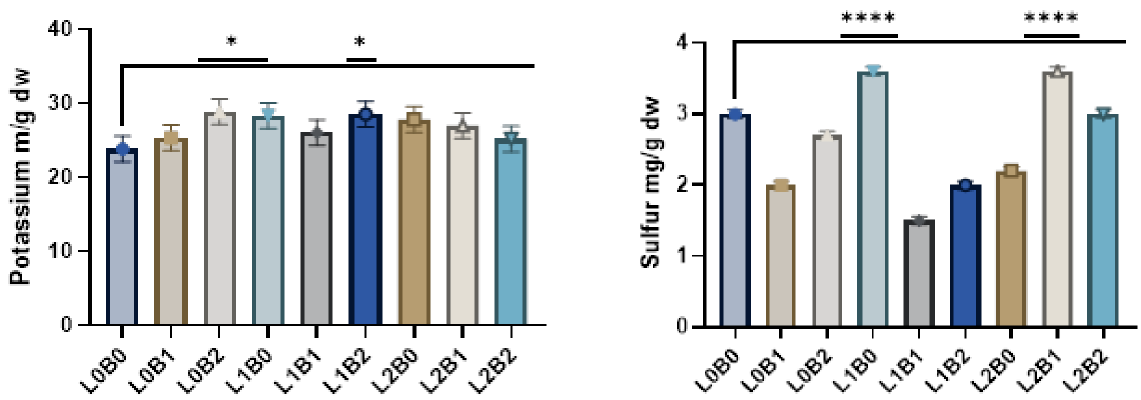


Figure (3): The effect of foliar application with Liquorice root extract (LRE) and Bread yeast extract (BYE) on macro elements content in Brassica curds. (A) Representative the effect LRE and BYE on the nitrogen content of Brassica curds. L0B0 control or untreated plants. L0B1treated plants with BYE at 10 g/L. L0B2 treated plants with BYE at 20 g/L. L1B0 treated plants with 10 g/L of LRE. L1B1treated plants with 10 g/L of LRE and 10 g/L of BYE. L1B2 treated plants with 10 g/L of LRE and 20 g/L of BYE. L2B0 treated plant with 20 g/L of LRE. L2B1 treated plants with 20 g/L of LRE and 10 g/L of BYE. L2B2 treated plants with 20 g/L of LRE and 20 g/L of BYE. (B) Representative the effect of LRE and BYE on the phosphor content of Brassica curds. (C) Representative the effect of LRE and BYE on the potassium content of Brassica curds. (D) Representative the effect of LRE and BYE on the sulfur content of Brassica curds. A multiple ANOVA was performed using ordinary one-way ANOVA multiple comparisons to compare the averages of treatments with L0B0. Significance was designated as follows: *$p < 0.05$, ****$p < 0.0001$.

GC-MS profile of methanolic Brassica curds extract of treated plants represents alteration in the active constituents compared to control plants (Table 2). The alteration may involve increase or decrease or disappearance of detected compounds area percentage according to applied treatments. For example, dimethyl trisulfide (compound is responsible for off-flavour in cooked Brassica vegetable) increased in curds of 10 g/L-treated plants and plants that sprayed with combination of 10 g/L and 20 g/L of LRE and BYE respectively. While compounds like phenol decreased in curds of sprayed plants with both biostimulants comparing to untreated plants. Saturated fatty acid Hexadecanoic acid and polyunsaturated fatty acid 9,12,15-Octadecatrienoic acid increased substantially in plants curds due to foliar application with biostimulants particularly with combination applications. Methyl ester compound such as Methyl 9-cis,11-trans-octadecadienoate was inexistent in untreated plants and gradually increasing with individual or combinated applications according to GC-MS profile. However, several compounds such as 4-Ethoxy-2-(methylamino) tropon, Furan-2-carboxaldehyde, 5-(1-piperidyl)- and gamma.-Sitosterol vanished in methanolic extract of Brassica curds due to influence of individual or combination applied treatments.

Table (2): GC-MS analysis profile of Brassica curds methanolic extract

R.T.	Compound name	Chemical class	L0B0	L0B1	L0B2	L1B0	L1B1	L1B2	L2B0	L2B1	L2B2
9.26	Dimethyl trisulfide	Organosulfur	3.68	3.58	3.13	4.06	2.87	4.98	2.81	2.79	2.89
18.43	Phenol, 4-ethenyl-2,6-dimethoxy-	Phenolic compounds	1.47	0.72	0.75	1.00	0.83	0.60	0.52	0.58	0.64
19.5	4-Ethoxy-2-(methylamino) tropone	Troponoid	8.48	5.79	6.16	-	-	4.10	5.29	5.84	-
	Furan-2-carboxaldehyde, 5-(1-piperidyl)-	Volatile compound	-	-	-	7.23	6.37	-	-	-	6.54
20.72	d-Proline	Amino acid	0.97	1.46	1.55	2.19	1.86	-	3.31	2.69	-
21.26	1H-Indole-3-acetonitrile	Glycosides	1.65	1.53	1.55	1.53	1.68	1.83	1.15	1.10	1.03
22.19	Hexadecanoic acid	Sat. Fatty acid	1.23	3.26	2.67	2.27	4.21	5.07	4.59	7.44	4.43
23.82	Methyl 9-cis,11-trans-octadecadienoate	Methyl ester	-	2.59	2.04	1.56	2.032	4.65	3.18	4.73	2.89
23.89	9,12,15-Octadecatrienoic acid	Unsat. Fatty acid	1.24	10.12	9.35	7.42	10.83	21.10	13.11	15.30	9.99
27.33	Glycerol monopalmitate	Fatty acid	1.33	1.18	1.51	1.57	2.18	1.38	2.18	1.73	0.86
33.29	.gamma.-Sitosterol	Sterol	0.45	0.51	0.76	2.11	-	-	1.93	2.00	1.53

4- Discussion

The utilization of botanical extract as biofertilizer or biostimulants to enhance plant growth or plant tolerance to stress conditions has gained considerable attention in recent years. Obtained results showed that carbohydrates content in curds decreased when foliar spray applied on Brassica plants which could be attributed to exploiting carbohydrates to increase the growth parameters of sprayed plants including plant high, leaves number, leaf area and biomass. Furthermore, total carbohydrates were utilized also by treated plants to increase the productivity traits such as yield, diameter, biomass, and weight of Brassica cruds [23]. Under similar conditions, instead of carbohydrates accumulation in induvial organs, plant direct total carbohydrates to promote its growth and yield [24]. On the other hand, protein percentage exhibited an increase in curds of L0B1-treated plants and L2B1-treated plants. The role of protein in the plant cover expanded ambit through their structural, enzymatic, metabolic, regulatic and storage functions [25]. Foliar application with biostimulants that are rich with protein such as BYE [26] or amino acid such as LRE [27] or both could increase the curds content of protein and promote yield quality from the healthy components.

Brassica curds are good source for phenolic compounds particularly flavonoids [14]. Phenols including flavonoids are natural compounds that are synthesized by the phenylpropanoid pathway and widely distributed in plants to promote and maintain its growth and development [28]. Besides, they have several medical benefits such as anticancer, antiinflammation and antioxidant properties [29]. In our study, the total of phenols and flavonoids in curds have not been shown significantly affected due to the foliar application with LRE or BYE. However, an insignificant increase was observed in phenols and flavonoids content in curds of L0B2-treated plants and L1B2-treated plants respectively. This increase may be related to the role of LRE and BYE in boosting and stimulating secondary metabolites production including total phenols and flavonoids, conforming with obtained outcomes by Younes in *Allium cepa* [30] and Dawood in *Glycine max* [31].

In the antioxidant context, previous literature indicated the active constituents in Brassicaceae vegetables and their related antioxidant properties [32]. The antioxidants activity in Brassica curds is correlated to various compounds such as phenols, flavonoids, sulfurs compounds, glucosinolates and others. This activity may be influenced extremely by several factors including cultivar, maturity, growing and harvesting conditions and storge circumstances [33]. Following the effect of LRE and BYE on antioxidant activity of curds methanol extract, L0B2-treated plant and L1B0-treated plants recorded a significant activity. Likewise, curds extract of plants sprayed with combination of both LRE and BYE concentrations recorded also antioxidant activity against DPPH. The augmentation of DPPH scavenging efficiency due to LRE implementing was observed previously in the extract of *Stevia rebaudiana bertoni* under normal conditions [34] and *Phaseolus vulgaris* L. under salinity conditions [17], both found LRE promoted antioxidant activity in plants due to increase the non-enzymatic antioxidant (e.g. proline, phenols, tocopherols, carotenoids, etc). In the current study, the capacity of curds extract to scavenging DPPH radicals could be increased regarding the increase of Dimethyl trisulfide, d-Proline, 9,12,15-Octadecatrienoic acid and gamma.-Sitosterol (Tab.1). Likewise, BYE application boosted antioxidants properties in *Allium sativum* L. as a tolerance strategy against drought stress through increasing of antioxidant enzymes and antioxidants compounds such as proline and ascorbic acid [35]. Our result indicated that BYE enhanced the scavenging of free radicals in curds extract of treated plants which could be imputed to induced role of BY in promoting compounds with antioxidants features including Dimethyl trisulfide, d-Proline, 9,12,15-Octadecatrienoic acid, Methyl 9-cis,11-trans-octadecadienoate, Glycerol monopalmitate and gamma.-Sitosterol. These compounds increased also in curds extract of sprayed plants with combination of LRE and BYE according to GC-MS analysis (Tab.1) which consequently enhanced its antioxidants efficiency

Microelements (N, P, K, S) increased compatibly with biostimulants foliar application. Brassica curds have been reported to contain a high macro elements content [36]. However, cultivars, growing and environmental conditions, and harvesting and post harvesting circumstances influence curds content of macro and micro elements [37, 38]. In a present study potassium recorded the highest content in Brassica curds and the content increased remarkably with LRE and BRE at L0B2 and L1B2-treated plants. Likewise, other macronutrients including N, P, and S increased in curds of treated plants: for N at L0B1 and L2B1, for P at L1B1, L2B0, and L2B1, and for S at L1B0 and L2B1. The increase of macro elements in curds may be referred to a rich content of elements in LRE [39] and BRE [40], these elements either move through or interact with the apoplast space which subsequently effect on their translocation to different plant parts including the curds [41]. Additionally, this increase may be linked to enhanced root capacity for nutrients uptake from the soil post biostimulants application [42]. It has been reported that LRE and BYE increased minerals content in mango fruit and potato tuber when biostimulants applied on the plants [43, 44]. Macro minerals almost participate in all human body functions and act significantly in metabolic processes. For example, phosphorus serve important role in the bone, teeth, phosphoproteins and nucleic acids formation, fat and starch metabolism and heartbeat and kidney function normalization [45].

GC-MS is mostly favoured technique to identify the quality and quantity of compounds in complex mixture due to its sensitivity and selectivity. Numerous agents influence GC-MS analysis results, some are related to plants (plants species, growing conditions, extraction methods) and others are related to GC-MS instrumentation conditions (temperature program, injection technique, detector sensitivity). Under our experimental conditions, GC-MS result of curds methanolic extract indicated high alteration in the peak area of most important detected compounds. Several compounds increased due to LRE or BYE or both applications such as Dimethyl trisulfide (L1B0, L1B2), d-Proline (L0B1, L0B2, L1B0, L1B1, L2B0, L2B1), 1H-Indole-3-acetonitrile (L1B1, L1B2), Hexadecanoic acid (all treatments), Methyl 9-cis,11-trans-octadecadienoate (all treatments), Glycerol monopalmitate (all treatment except L2B2) and gamma.-Sitosterol (L0B1, L0B2, L1B0, L2B0, L2B1, L2B2). On the contrary, several compounds decreased due to individual or combination of foliar applications such as Dimethyl trisulfide (L0B1, L0B2, L1B0, L1B1, L2B0, L2B1, L2B2), Phenol, 4-ethenyl-2,6-dimethoxy- (all treatments), 4-Ethoxy-2-(methylamino) tropone (L0B1, L0B2, L1B2, L2B0, L2B1), 1H-Indole-3-acetonitrile (L0B1, L0B2, L1B0, L2B0, L2B1, L2B2), and Glycerol monopalmitate (L2B2). Interestingly, some compounds disappeared due to LRE or BYE or both applications such as 4-Ethoxy-2-(methylamino) tropone (L1B0, L1B1, L2B2), Furan-2-carboxaldehyde, 5-(1-piperidyl)- (L0B0, L0B1, L0B2, L1B2, L2B0, L2B1), d-Proline (L1B2, L2B2), and gamma.-Sitosterol (L1B1, L1B2). The alteration of active compounds in Brassica curds extract with LRE foliar spray may be correlated to rich content of LRE from sugars, vitamins, mineral nutrients, amino acids, selenium, and phytohormones which effect on secondary metabolites biosynthesis pathways such as sulfurs-containing compounds pathway [46], shikimate/phenylpropanoid pathway [47], hormone biosynthesis pathways [48], and sterol biosynthesis pathway [49]. Likewise, BYE application caused a variation in peak areas of secondary compounds in Brassica curds extract which may be referred to the BYE content of effective components including amino acids, vitamins, proteins and hormones [50]. Some of these components serve as precursors for secondary metabolites biosynthesis pathways such as amino acids [51], while some act as enzymes cofactors, signaling molecules, and genes expression regulators such as vitamins and hormones [52, 53]. These active compounds in Brassica curds have crucial role in maintaining human health when incorporated into the daily dietary due to their therapy functions as antioxidants, antiinflammation, anticancer, antiviral, anti-obesities, antihypertensive, and antigenotoxicity [54]. For example, 1H-Indole-3-acetonitrile displayed a broad-spectrum antiviral potential against influenza A, HSV-1, VSV and SARS-CoV-2 [55, 56]. Thus, the increase of their amount after LRE and BYE application may enhance the nutritional, health-promoting and therapeutic value of brassica curds. The Two-Way ANOVA analysis revealed that both LRE and BYE individually influenced most physiological and biochemical parameters ($p < 0.05$). However, the significant interaction term (LRE × BYE) for several key traits—such as DPPH scavenging activity, protein concentration, macronutrients content and some active constituents —indicates a synergistic response rather than a simple additive effect. This suggests that the combined biostimulants treatment enhances metabolic coordination, leading to greater metabolic productions compared to individual applications. Thus, the improved plant performance under combined treatments can be attributed to the integrated modulation of stress physiology driven by synergistic biochemical interactions between LRE and BYE.

5- Conclusion

In summary, we found that the combination of biostimulants including LRE and BYE could be affected prominently on metabolites content of Brassica curds. When two biostimulants are applied as combination, their act may complement each other or act synergistically to enhance the overall effect, resulting in improving plants yield quantity and quality in the region that has desert soil properties. Our results indicated an increase in primary and secondary metabolites in Brassica curds after foliar application of LRE and BYE concentrations. Although the induvial treatments had an obvious impact on experimental parameters, the combination of study agents also acted synergistically to boost these parameters. In crops cultivation under desert conditions, obtained findings could be considered a promising result within the sustainable required direction. The remarkable function of LRE and BYE in improving the quality components of Brassica curds including phenols, flavonoids, macronutrients, and active compounds provides compelling evidence of the alternative role that biostimulants may play in eco-friendly agriculture. This improvement not only increase the nutritional value of Brassica curds but also enrich them with active compounds that contribute to the promoting of healthy foods sources.

Author contribution

Z.A designed research studies and performed field work, W.N and F.H performed lab experiments and data analysis. W.N, F.A, Y.K.A, V.R., P.S, A.M, S.V, and V.R. PB- writing, review and edited the manuscript. Funding acquisition-W.N. All authors have read and approved the final version of the manuscript

Declarations

Availability of data and materials: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author

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