

Original Research Article

Improving the leaf nutrient and Mineral Elements of Constituents (*Moringa oleifera* L.) by Application of Certain Biostimulants and Biofertilizers

ABSTRACT

Increasing the need for food as a result of global population growth negatively impacted the ecology and soil. As a result of an increase in the demand for industrial chemical fertilizers and inefficient crop management. In this research, the effect of some biofertilizers, amino acids, and seaweed extracts on the chemical composition of the leaves of *Moringa* plants was evaluated. The results showed that bio-fertilization with phosphorine biofertilizers associated with amino acids and seaweed extracts at 700 mg/L (T25) was the most effective treatment for improving and maximizing the leaf nitrogen content, with a mean value for both seasons of 427.3 ml/100 g. Also Herein, T26 (soil bio-fertilized with cerealine plus bio-fertilizer with amino acid and seaweed extracts as foliar spray each at 700 mg/L) was the most effective treatment in improving that reflected the peak of leaf contents of phosphorus, with a mean value of 197.7 ml/100 g, leaf potassium content with mean values 1.379 ml/100 g, leaf calcium content with a mean value of 2.355 mg/L, vitamin C content with a mean value 17.49 ml/100g and leaf total carbohydrates content with a mean values (34.66 ml/100 g for both seasons was observed with those plants that were soil bio-fertilized with cerealine bio-fertilizer with amino acid and seaweed extracts foliar spray each at 700 mg/L (T26) which produced a better response than using each treatment separately.

Key words: *Moringa oleifera*, biofertilizers, phosphorine, cerealine, amino acids, seaweed extract, vitamin C, total carbohydrates

INTRODUCTION

Moringa oleifera Lam (syn. *M.ptreygosperma* Gaertn), globally recognized as a 'superfood' due to its high nutritional content, *M. oleifera* is rich in phytochemicals and bioactive organic substances [1]. *Moringa oleifera* is one of 13 species belonging to the genus *Moringa*, which is the only genus in the Moringaceae family, originating from India and spreading in Africa, especially in Ethiopia, Kenya, Sudan and Egypt, and it grows in the tropics [2]. *Moringa* plant can grow in all types of soils, but loam and clay loam soils are optimal for its cultivation [3]. In recent years, researchers around the world have paid much attention to the *Moringa* tree because of its great nutritional and medical importance, which makes it have a major role in raising the global and local economic level for its producing countries [4 and 5]. It can be established like a crop, living fence, continuous windbreaker, and agroforestry system. It tolerates extreme pruning and has excellent regrowth capacity [6]. It can be consumed as tea, fresh, dry, tablets, capsules, or a vegetable that can be added to the typical foods of each region [7]. The nutritional value of the leaves can be used to complement the nutrition of human people and animals [8]. The nutritional content gives it a great nutritional and medicinal potential that can be used to treat or prevent different types of nutritional diseases [9]. By the phytochemical analysis of *M. oleifera*, researchers from different countries have shown that the leaves are particularly rich in potassium, calcium, phosphorus, iron, vitamin D, essential amino acids, as well as known antioxidants such as carotene, vitamin C, and flavonoids [10].

Moringa leaves contain large amounts of nutrients, and being a perennial species, they are available throughout the year [11 and 12]. For this reason, organizations such as FAO, promote its cultivation and consumption [13 and 14]. In recent decades, the need to meet the growing demand for food due to the increase in population has led to the demand for the consumption of agricultural products, [15]. In this quest to improve the production rate, elemental nutrients, such as phosphorus, potassium, and nitrogen were supplied to soils by the application of chemical fertilizers [16]. Fertilizers increase crop yields; however, it has been observed that the constant use of chemical-based fertilizers affects the quality of

soils and poses a health risk to consumers [17]. Therefore, producers currently prefer to change production techniques towards green agricultural practices with the use of new biotechnologies, such as the use of biofertilizers [18]. Biofertilization has emerged as a possible substitute for chemical fertilizers in terms of cutting down on sources of environmental pollution. In addition to chemical and organic fertilizers, it involves adding inoculants and biological extracts to the soil, seeds, seedlings, or leaves. Biological fertilizers cannot be used as a substitute for chemical fertilizers, but rather they are supplementary fertilizers for mineral fertilization, as they contribute into increasing the effectiveness and efficiency of chemical fertilizers [19 and 20]. The nutritional and functional qualities are affected by multiple factors, and when ignored, they limit the maximum nutritional use of the leaves [21 and 22]. Several previous studies indicated the effect of different types of bio-fertilizers and the role and mechanism these fertilizers play in their impact on plant growth. Shareef[23] pointed out the importance of using bio-fertilizers in providing some of the important nutrients for the plant, such as nitrogen, phosphorous, and potassium, as well as the secretion of some hormones and acids that act as plant growth regulators. While, Al Dayel [24] confirmed the existence of a significant difference when using biofertilizer, which increases the percentage of nitrogen, which is an important element to increase vegetative growth in the Moringa plant. To maximize the production and nutritional use of the Moringa plants in Egypt, there is a need to understand the effect of some biofertilizers, amino acids, and seaweed extracts on the nutritional qualities of this Moringa plants. As far as we know, there is little scientific literature about the multiple factors that affect the nutritional quality of moringa leaves. Therefore, the objective of this study was to identify the effects of some biofertilizers, amino acids, and seaweed extracts on the nutritional content of *M. oleifera* leaves, and reduce the intensive use of chemical fertilizers.

MATERIALS AND METHODS

Two field experiments were carried out at the Faculty of Agriculture (Saba Basha), Alexandria University's experimental farm in the Abees district of Alexandria governrate, Egypt, during the 2020 and 2021 growing seasons. The

experiments were designed to assess the effects of soil bio-fertilization (Phosphorine and cerealine), spraying with varying rates of amino acids, and seaweed extract on the chemical composition of moringa leaves (*Moringa oleifera* L.).

The trials' soil was tilled, split into rows and plots, and then planted in 2.5 × 2.5m-meter sections in March. All research plots received the same applications of mineral fertilizers: 600 g/tree of ammonium nitrate (33.5% N), 250 g/tree of calcium superphosphate (15.5% P₂O₅), and 300 g/tree of potassium sulfate (48% K₂O). One dose of calcium superphosphate was mixed into the top soil at a depth of 0.15 meters around the tree trunk. Three equal doses of nitrogen and potassium fertilizer were applied; the first dose was given after 20 days of seeding???, the second dose was given after the first cut, and the third dose was given after the second cut. Furthermore, biofertilizers, such as Phosphorine (containing P-dissolving bacteria such as *Bacillus* sp.) and cerealine (produced by the Egyptian Ministry of Agriculture and containing *Bacillus polymyxa* and *Azotobacter chroococcum* bacteria at 107 colony-forming units (CFU)/g carrier), wereare also used as nitrogen sources. And amino acids and seaweed extract (0, 350, and 700 mg/l) were administered as a foliar spray three times: after planting, after the first cut, and after the second onecut. Control plants were doused with water. The plants were harvested three times during the first and second seasons by cutting the vegetative sections to get the nutritional quality of moringa leaves data.

The following characteristics were measured in the dry leaves hours at the harvest: leaf nitrogen content [25], leaf phosphorus content [26], leaf potassium content [26], leaf calcium content [26], vitamin C [27], and total carbohydrates [28].

Statistical analysis

The split-split plot design was used to arrange the 27 treatments, with each treatment being repeatedroduced three times. All data collected throughout the two research seasons were statistically examined using the analysis of variance approach described by [29]. The least significant differences test (L.S.D.) at 0.05 was used to compare the means of the various treatments.

Where???:

Whole plot

Subplot

Sub-subplot

These need 6 values for LSD test for A, B, C, AB, AC, ABC.

RESULTS AND DISCUSSION

Leaf nitrogen content.

With respect to leaf nitrogen content of *M. oleifera* plants as affected by the different investigated treatments, data presented in **Table 1** indicated that bio-fertilization with phosphorine biofertilizers associated with amino acid and seaweed extracts each at **700 mg/L (T25)** was the most effective treatment in enhancing and maximizing leaf nitrogen content, with a mean value for both seasons of 427.3 mg/100g, followed by spraying with amino acid and seaweed extracts at 700 mg/L and cerealine bio-fertilizer (T₂₆) with a mean value for both seasons of 421.4 mg/100g, which ranked second in the frame of treatments evaluation. While treatment with each individual, phosphorine (T₁) treatment (T₁) alone gave a mean leaf nitrogen content of 258.8 mg/100g, seaweed alone (T₆) gave 284.6 mg/100g, and amino acid alone (T₁₈) gave 356.8 mg/100g. On the other hand, the control plants (T₀) contained revealed the lowest value of leaf nitrogen content of 6.92 mg/100g for both seasons. Data showed that spraying with amino acids and seaweed extracts with soil bio-fertilization produced a better response than using each component separately.

The findings are consistent with those of [30 and 31], who found that spraying seaweed extract had a beneficial effect on the mineral contents; furthermore, they claimed that plants treated with seaweed spray had mineral levels that were noticeably greater than those of untreated plants. Moreover, Mazher [32] observed that Phosphorine biofertilizers enhanced the nitrogen content of *M. oleifera* in

leaves and stems when compared to the control. Also, Radwan [33] found that bio-fertilization enhanced the chemical composition of nitrogen percentage when applied to moringa plants. In another study, Abd El-baset [35] showed that using high concentration of amino acids as foliar sprays on both *Moringa oleifera*, and *Moringa ovalifolia* plants gave the highest amount of leaf nitrogen content. Also, Haider [36] reported that foliar spraying of seaweed extract improved the nitrogen content of potato tubers.

UNDER PEER REVIEW

Table (1): Means of Leaf Nitrogen content of *Moringa oleifera* as affected by Bio-fertilizers, Amino acids, and Seaweed extracts and their combinations during 2020 and 2021 growing seasons.

Treatments	Leaf Nitrogen (mg/100g)						Mean
	Season2020			Season2021			
	1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut	
T ₀ Untreated	295.1	293	220.2	224.8	252.5	254.2	256.6
T ₁ Phosphorein	306.1	304	218.9	215.5	253.9	254.6	258.8
T ₂ Cerealine	310.7	304.2	223.7	232.5	253.5	253.5	263.0
T ₃ Seaweed 350mg/l	298.3	309.8	236.4	255.9	271.4	259.9	271.9
T ₄ Seaweed 350mg/l + Phosphorein 350mg/l	321.9	317.1	233.3	241.3	269.7	265.1	274.7
T ₅ Seaweed 350mg/l + Cerealine 350mg/l	317.4	320.6	254.1	254.8	263.8	258.1	278.1
T ₆ Seaweed 700mg/l	315.9	305.7	277.8	262.7	271.5	274	284.6
T ₇ Seaweed 700mg/l + Phosphorein 700mg/l	324.6	315	285.6	276.9	266.4	266.5	289.2
T ₈ Seaweed 700mg/l + Cerealine 700mg/l	345.3	321.3	291.6	259.8	232.6	246.9	282.9
T ₉ Amino 350mg/l	298.5	307.2	306.3	311	274.1	242.4	289.9
T ₁₀ Amino 350mg/l + Phosphorein	329.5	324.7	309.4	307.8	289.6	290.1	308.5
T ₁₁ Amino 350mg/l + Cerealine	341.9	322.4	306.7	309.5	281.9	273.2	305.9
T ₁₂ Amino 350mg/l + Seaweed 350mg/l	338.5	308.3	335.5	309.3	286.2	280.6	309.7
T ₁₃ Amino 350mg/l + Seaweed 350mg/l + Phosphorein	347.3	342.2	344.9	316.3	296.3	285.9	322.2
T ₁₄ Amino 350mg/l + Seaweed 350mg/l + Cerealine	342.2	354.9	348.1	330.9	303.7	332.9	335.5
T ₁₅ Amino 350mg/l + Seaweed 700mg/l	313.9	321.8	350.3	332.7	282.5	292.7	315.7
T ₁₆ Amino 350mg/l + Seaweed 700mg/l + Phosphorein	354.9	363.3	356.5	335.7	307.9	307	337.6
T ₁₇ Amino 350mg/l + Seaweed 700mg/l + Cerealine	339.5	365.6	390.7	348.15	314.4	306	344.1
T ₁₈ Amino acid 700mg/l	395.4	308.5	414	383.8	326.7	312.5	356.8
T ₁₉ Amino acid 700mg/l + Phosphorine	411.6	414.5	413.3	420.2	356.9	345.9	393.7
T ₂₀ Amino acid 700mg/l + Cerealine	413.7	420.2	417.7	423.5	289	367.6	388.6
T ₂₁ Amino acid 700mg/l + Seaweed extract 350mg/l	404.4	352	415	433.8	393.7	386.6	397.6
T ₂₂ Amino acid 700mg/l + Seaweed extract 350mg/l + Phosphorine	417.5	420.5	417.7	436.9	414.9	402.2	418.3
T ₂₃ Amino acid 700mg/l + Seaweed extract 350mg/l + Cerealine	417.9	424.9	415	437.6	414.5	413.8	420.6
T ₂₄ Amino acid 700mg/l + Seaweed extract 700mg/l	415.7	355.8	418.3	439	419.7	418.4	411.2
T ₂₅ Amino acid 700mg/l + Seaweed extract 700mg/l + Phosphorine	418.1	425.8	424.6	440.9	422.6	431.6	427.3
T ₂₆ Amino acid 700mg/l + Seaweed extract 700mg/l + Cerealine	413.1	412.8	418.2	440.8	421.2	422.5	421.4
L.S.D	18.5	57.1	12.9	29.2	4.02	29.6	

The L.S.D needs 6 values for LSD 5%

test for A, B, C, AB, AC and ABC.



UNDER PEER REVIEW

Leaf phosphorus content.

Data concerning the relationship between the leaf phosphorous content of *M. oleifera* plants and the different investigated treatments are tabulated in **Table 2**. The data display that the investigated parameter was significantly responded to the different studied treatments. Herein, the most effective treatment that reflected the peak of leaf phosphorous content was observed with those plants that were soil bio-fertilized with cerealine bio-fertilizer with amino acid and seaweed extracts foliar spray each at 700 mg/L (T26) with a mean value for both seasons of 197.7 ml/100 g, followed by spraying with amino acid and seaweed extracts at 700 mg/L, and Phosphorine bio-fertilizer (T25) with a mean value for both seasons of 195.3 ml/100 g, came in the second rank in this respect. While that treatment with each individual, cerealine (T2) treatment alone gave a mean leaf phosphorous content of 172.8 ml/100 g, seaweed alone (T6) gave 171 ml/100g, and amino acid alone (T18) gave 182.5 ml/100 g. On the other hand, the control (untreated plants) (T0) revealed the lowest mean value of leaf phosphorous content of 170.5 ml/100 g for both seasons. Based on the data, spraying amino acid and seaweed extracts with soil bio-fertilization led to better results than utilizing each component alone. The results are in tone with the findings of Zhang [37], who claimed that the foliar application of seaweed extract plays a significant function in promoting cell membrane. Likewise, Radwan [33] found that bio-fertilization enhanced the chemical composition of phosphorus percentage when applied to moringa plants. Zayed [34] demonstrated that *M. oleifera* seeds were grown in soil inoculated with mixed cultures of *Saccharomyces cerevisiae*, *Azotobacter chroococcum*, *Bacillus circulans*, and *Azot. chroococcum*, which produced the highest levels of phosphorous. Likewise, Abd El-baset [35] showed that using high concentration of amino acids as foliar sprays on both *M. oleifera*, and *M. ovalifolia* plants gave the highest amount of leaf phosphorus content.

Table (2): Means of Leaf Phosphorus content of *Moringa oleifera* as affected by Bio-fertilizers, Amino acids, and Seaweed extracts and their combinations during 2020 and 2021 growing seasons.

Treatments	Leaf Phosphorus (ml/100g)						Mean
	Season2020			Season2021			
	1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut	
T ₀ Untreated	170.3	166.9	170.6	170.9	170.6	173.7	170.5
T ₁ Phosphorein	177.6	173.6	167.9	171.6	170.4	171.4	172.1
T ₂ Cerealine	175.1	178	165.9	173.8	171.3	172.8	172.8
T ₃ Seaweed 350mg/l	177.6	166.9	168.5	173.5	169.9	174.4	171.8
T ₄ Seaweed 350mg/l + Phosphorein 350mg/l	178.4	177.6	164.8	171.2	172.3	175.3	173.3
T ₅ Seaweed 350mg/l + Cerealine 350mg/l	180.3	177.4	171.9	175.9	171.1	173.8	175.1
T ₆ Seaweed 700mg/l	183.5	162.7	166.9	170.4	170.9	171.7	171.0
T ₇ Seaweed 700mg/l + Phosphorein 700mg/l	186.2	177.5	172	172.1	171.3	171.4	175.1
T ₈ Seaweed 700mg/l + Cerealine 700mg/l	173.8	179.5	171.3	174.2	171.2	174.7	174.1
T ₉ Amino 350mg/l	176.3	158.9	172.0	170.8	171.1	173.3	170.4
T ₁₀ Amino 350mg/l + Phosphorein	175.4	175.5	173	172.4	176	172.4	174.1
T ₁₁ Amino 350mg/l + Cerealine	174.7	177.8	175.8	172.9	173.5	174.0	174.8
T ₁₂ Amino 350mg/l + Seaweed 350mg/l	177.2	165.4	187.5	176.3	179.5	172.7	176.4
T ₁₃ Amino 350mg/l + Seaweed 350mg/l + Phosphorein	178.9	179.5	184.9	177.7	180.5	180.4	180.3
T ₁₄ Amino 350mg/l + Seaweed 350mg/l + Cerealine	180.4	183.6	188.2	177.9	180.6	180.3	181.8
T ₁₅ Amino 350mg/l + Seaweed 700mg/l	180.4	180.2	188.3	179.9	181.6	183.6	182.3
T ₁₆ Amino 350mg/l + Seaweed 700mg/l + Phosphorein	183	186.2	179	181	183.6	188.5	183.6
T ₁₇ Amino 350mg/l + Seaweed 700mg/l + Cerealine	182.1	190.2	178.1	180	181.5	185.1	182.8
T ₁₈ Amino acid 700mg/l	183.0	179.9	184.5	182.4	180.5	184.5	182.5
T ₁₉ Amino acid 700mg/l + Phosphorine	184	183.1	186.2	187.7	188.5	185.8	185.9
T ₂₀ Amino acid 700mg/l + Cerealine	184.7	181.9	185.3	189.2	187.9	186.5	185.9
T ₂₁ Amino acid 700mg/l + Seaweed extract 350mg/l	185.8	186.1	196.5	192.9	190.3	187.2	189.8
T ₂₂ Amino acid 700mg/l + Seaweed extract 350mg/l + Phosphorine	186.2	188.4	196.7	194.7	195.9	194.3	192.7
T ₂₃ Amino acid 700mg/l + Seaweed extract 350mg/l + Cerealine	188.4	191.3	191.2	193.5	195.4	196.2	192.7
T ₂₄ Amino acid 700mg/l + Seaweed extract 700mg/l	190.7	192.2	195.9	196.8	194.2	194.3	194.0
T ₂₅ Amino acid 700mg/l + Seaweed extract 700mg/l + Phosphorine	194.4	193.9	194.3	196.9	194.4	198.1	195.3
T ₂₆ Amino acid 700mg/l + Seaweed extract 700mg/l + Cerealine	197.6	197.7	197.8	199	198.9	195.2	197.7
L.S.D	2.09	4.26	4.42	4.26	4.22	5.92	

Leaf potassium content.

Data in **Table 3** clearly show that the peak of leaf potassium content was detected with either those sprayed plants with amino acid and seaweed extracts at 700 mg/L with soil bio-fertilized cerealine bio-fertilizer (T₂₆), which achieved the highest significant mean values 1.379 ml/100g of leaf potassium content for both seasons, followed by soil biofertilization phosphorine with foliar application of amino acids and seaweed extracts at 700 mg/L (T₂₅) with a mean value for both seasons of 1.374 ml/100 g, came in the second rank in this respect. While that treatment with each individual, cerealine (T₂) treatment alone gave a mean leaf potassium content of 0.600 ml/100 g, seaweed alone (T₆) gave 0.924 ml/100g, and amino acid alone (T₁₈) gave 1.316 ml/100 g. On the other hand, the control (untreated plants) (T₀) revealed the lowest mean value of leaf potassium content of 0.530 ml/100 g for both seasons. Based on the data, foliar spraying seaweed extracts and amino acids with soil bio-fertilization produced greater results than using each component separately. These findings are in accordance with Abd El-baset [35] showed that using high concentration of amino acids as foliar sprays on both *M. oleifera*, and *M. ovalifolia* plants gave the highest amount of leaf potassium content. Likewise, Mazher [32] reported that phosphorine biofertilizers increased the potassium content of *M. oleifera* in leaves and stems as compared with the control. Yang [38] reported that seaweed extracts increased the levels of potassium, in apples. Also, Radwan [33] found that bio-fertilization enhanced the chemical composition of potassium percentage when applied to moringa plants. As indicated by El-Leithy [39], nitroben and phosphorein both raised potassium percentages in the herb compared to the control in the first and second cuttings of the first and second seasons.

Table (3): Means of Leaf Potassium content of *Moringa oleifera* as affected by Bio-fertilizers, Amino acids, and Seaweed extracts and their combinations during 2020 and 2021 growing seasons.

Treatments	Leaf Potassium (mg/100g)						Mean
	Season2020			Season2021			
	1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut	
T ₀ Untreated	0.734	0.629	0.413	0.578	0.414	0.414	0.530
T ₁ Phosphorein	0.904	0.619	0.538	0.574	0.452	0.497	0.597
T ₂ Cerealine	0.899	0.617	0.556	0.575	0.485	0.469	0.600
T ₃ Seaweed 350mg/l	0.944	0.753	0.764	0.637	0.687	0.472	0.709
T ₄ Seaweed 350mg/l + Phosphorein 350mg/l	0.937	0.648	0.838	0.748	0.546	0.573	0.715
T ₅ Seaweed 350mg/l + Cerealine 350mg/l	0.952	0.727	0.940	0.775	0.897	0.788	0.847
T ₆ Seaweed 700mg/l	1.002	0.718	0.921	0.916	0.882	1.103	0.924
T ₇ Seaweed 700mg/l + Phosphorein 700mg/l	1.11	0.758	1.01	1.190	1.102	1.103	1.046
T ₈ Seaweed 700mg/l + Cerealine 700mg/l	1.13	0.906	1.12	1.183	1.104	0.937	1.063
T ₉ Amino 350mg/l	1.20	1.36	1.13	1.263	0.951	0.427	1.055
T ₁₀ Amino 350mg/l + Phosphorein	1.18	0.798	1.19	1.285	1.124	1.125	1.117
T ₁₁ Amino 350mg/l + Cerealine	1.24	1.28	1.24	1.274	1.206	1.191	1.239
T ₁₂ Amino 350mg/l + Seaweed 350mg/l	1.22	1.26	1.24	1.283	1.144	1.142	1.215
T ₁₃ Amino 350mg/l + Seaweed 350mg/l + Phosphorein	1.23	1.27	1.23	1.328	1.144	1.149	1.225
T ₁₄ Amino 350mg/l + Seaweed 350mg/l + Cerealine	1.23	1.24	1.235	1.345	1.142	1.143	1.223
T ₁₅ Amino 350mg/l + Seaweed 700mg/l	1.235	1.204	1.253	1.318	1.237	1.222	1.245
T ₁₆ Amino 350mg/l + Seaweed 700mg/l + Phosphorein	1.246	1.279	1.253	1.34	1.331	1.345	1.299
T ₁₇ Amino 350mg/l + Seaweed 700mg/l + Cerealine	1.260	1.257	1.249	1.329	1.326	1.319	1.290
T ₁₈ Amino acid 700mg/l	1.314	1.255	1.349	1.391	1.268	1.318	1.316
T ₁₉ Amino acid 700mg/l + Phosphorine	1.312	1.302	1.362	1.449	1.338	1.338	1.350
T ₂₀ Amino acid 700mg/l + Cerealine	1.305	1.266	1.349	1.463	1.311	1.311	1.334
T ₂₁ Amino acid 700mg/l + Seaweed extract 350mg/l	1.319	1.302	1.349	1.465	1.224	1.230	1.315
T ₂₂ Amino acid 700mg/l + Seaweed extract 350mg/l + Phosphorine	1.347	1.334	1.354	1.475	1.391	1.376	1.379
T ₂₃ Amino acid 700mg/l + Seaweed extract 350mg/l + Cerealine	1.336	1.344	1.362	1.476	1.314	1.316	1.358
T ₂₄ Amino acid 700mg/l + Seaweed extract 700mg/l	1.308	1.311	1.332	1.454	1.252	1.34	1.333
T ₂₅ Amino acid 700mg/l + Seaweed extract 700mg/l + Phosphorine	1.312	1.356	1.318	1.469	1.369	1.421	1.374
T ₂₆ Amino acid 700mg/l + Seaweed extract 700mg/l + Cerealine	1.339	1.382	1.344	1.464	1.392	1.356	1.379
L.S.D	0.11	0.23	0.06	0.17	5.85	2.85	

Leaf calcium content.

Data dealing with the response of leaf calcium content to the different investigated treatments are presented in **Table 4**. Such recorded data obviously display that leaf calcium content significantly responded to the studied treatments. Furthermore, soil bio-fertilization Cerealine bio-fertilizer with either amino acid and seaweed extracts at 700 mg/L (T₂₆) maximized leaf calcium content with a mean value of 2.355 mg/L for both seasons, followed by soil bio-fertilizer Phosphorine with either amino acid and seaweed extracts at 700 mg/L (T₂₅) arranged in the second rank. While that treatment with each individual, cerealine (T₂) treatment alone gave a mean leaf calcium content of 0.144 ml/100 g, seaweed alone (T₆) gave 0.302 ml/100 g, and amino acid alone (T₁₈) gave 1.626 ml/100 g. On the other way around, the minimum value of such an investigated parameter (leaf calcium content) was associated with untreated plants (control-T₀), with a mean value of leaf calcium content of 0.530 ml/100 g for both seasons. Based on the data, foliar spraying seaweed extracts and amino acids with soil bio-fertilization produced greater results than using each component separately. Our results dealing with the beneficial impact of bio-fertilizers on enhancing the leaf nutritional status of *M. oleifera* plants are supported by the early findings of some investigators. As demonstrated by Ghoneim and EL-Araby [40] who reported some enhancing effects on the concentrations of calcium in jew's mallow leaves as a result of inoculation either with Nitrobenbiofertilizer. Also, Youssef [41] reported that applying biofertilizer treatments to *M. oleifera* plants resulted in higher leaf calcium levels compared to the control. Likewise, Abd El-baset [35] showed that using high concentration of amino acids as foliar sprays on both *Moringa oleifera*, and *Moringa ovalifolia* plants gave the highest amount of leaf calcium content. Moreover, previous research has demonstrated that applying seaweed extract to plants' leaves enhances nutrient absorption and raises the concentrations of macro- and micronutrients such as calcium in the plants [42, 31 and 38].

Table (4): Means of Leaf Calcium content of *Moringa oleifera* as affected by Bio-fertilizers, Amino acids, and Seaweed extracts and their combinations during 2020 and 2021 growing seasons.

Treatments	Leaf Calcium (mg/100g)						Mean
	Season2020			Season2021			
	1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut	
T ₀ Untreated	0.08	0.08	0.36	0.06	0.09	0.09	0.13
T ₁ Phosphorein	0.08	0.08	0.08	0.09	0.06	0.12	0.09
T ₂ Cerealine	0.08	0.15	0.08	0.09	0.24	0.20	0.14
T ₃ Seaweed 350mg/l	0.09	0.19	0.08	0.18	0.39	0.39	0.22
T ₄ Seaweed 350mg/l + Phosphorein 350mg/l	0.09	0.35	0.08	0.15	0.09	0.38	0.19
T ₅ Seaweed 350mg/l + Cerealine 350mg/l	0.08	0.38	0.08	0.16	0.25	0.36	0.22
T ₆ Seaweed 700mg/l	0.08	0.18	0.11	0.64	0.33	0.44	0.30
T ₇ Seaweed 700mg/l + Phosphorein 700mg/l	0.09	0.36	0.18	0.88	0.09	0.51	0.35
T ₈ Seaweed 700mg/l + Cerealine 700mg/l	0.09	0.10	0.28	0.89	0.98	0.53	0.48
T ₉ Amino 350mg/l	0.39	0.14	0.81	0.60	0.08	0.94	0.50
T ₁₀ Amino 350mg/l + Phosphorein	0.69	0.17	0.71	0.95	1.08	0.91	0.75
T ₁₁ Amino 350mg/l + Cerealine	0.66	0.12	0.87	1.00	1.10	0.89	0.77
T ₁₂ Amino 350mg/l + Seaweed 350mg/l	1.41	1.31	1.30	1.13	1.21	1.22	1.26
T ₁₃ Amino 350mg/l + Seaweed 350mg/l + Phosphorein	1.55	1.41	1.35	1.32	1.20	1.34	1.36
T ₁₄ Amino 350mg/l + Seaweed 350mg/l + Cerealine	1.62	1.60	1.40	1.41	1.35	1.46	1.47
T ₁₅ Amino 350mg/l + Seaweed 700mg/l	1.83	1.70	1.59	1.67	1.24	1.24	1.55
T ₁₆ Amino 350mg/l + Seaweed 700mg/l + Phosphorein	1.93	1.74	1.74	1.82	1.56	1.20	1.67
T ₁₇ Amino 350mg/l + Seaweed 700mg/l + Cerealine	1.97	1.89	1.81	1.91	1.69	1.27	1.76
T ₁₈ Amino acid 700mg/l	1.91	1.93	1.20	1.92	1.25	1.52	1.62
T ₁₉ Amino acid 700mg/l + Phosphorine	1.93	1.90	1.61	2.11	1.80	1.85	1.87
T ₂₀ Amino acid 700mg/l + Cerealine	2.00	1.96	1.75	2.12	2.00	1.91	1.96
T ₂₁ Amino acid 700mg/l + Seaweed extract 350mg/l	2.00	1.95	2.00	2.00	2.02	2.39	2.06
T ₂₂ Amino acid 700mg/l + Seaweed extract 350mg/l + Phosphorine	2.03	2.07	2.00	2.04	2.01	2.08	2.04
T ₂₃ Amino acid 700mg/l + Seaweed extract 350mg/l + Cerealine	2.67	2.00	2.00	2.11	2.01	2.37	2.19
T ₂₄ Amino acid 700mg/l + Seaweed extract 700mg/l	2.00	2.14	2.00	2.07	2.00	2.37	2.09
T ₂₅ Amino acid 700mg/l + Seaweed extract 700mg/l + Phosphorine	3.00	2.02	2.00	2.11	2.16	2.26	2.26
T ₂₆ Amino acid 700mg/l + Seaweed extract 700mg/l + Cerealine	3.00	2.13	2.04	2.72	2.10	2.14	2.35
L.S.D	0.16	0.21	0.36	0.37	.037	2.78	

Vitamin C

Data concerning leaf vitamin C content are presented in **Table 5**, such data display that the optimum value of such a parameter was recorded with cerealine soil bio-fertilization and either amino acid and seaweed extract foliar spray each at 700 mg/L (T₂₆) with a mean value for both seasons of 17.49 ml/100g, followed by spraying with amino acid and seaweed extracts at 700 mg/L and Phosphorine bio-fertilizer (T₂₅) with a mean value for both seasons of 17.34 ml/100 g, which ranked second in the frame of treatment evaluation, while that treatment with each individual, where cerealine (T₂) treatment alone gave a mean vitamin C content of 14.22ml/100g, seaweed alone (T₆) gave 14.32ml/100g, and amino acid alone (T₁₈) gave 14.85ml/100g. On the other way around, the untreated plants (control) (T₀) revealed the lowest value of leaf vitamin C of 14.14 ml/100 g for both seasons. Data showed that spraying with amino acid and seaweed extracts with soil bio-fertilization produced a better response than using each component separately. In light of this, several reports indicated that Radwan [33] found that bio-fertilization enhanced the chemical composition of vitamin C **percentage** when applied to moringa plants. Also, Radwan [43] demonstrated that all treatments, including biofertilization cerealine and phosphorein on fertilization, enhanced the chemical composition, including vitamin C content, of the leaves of the parsley plant. In the same direction. Furthermore, Abd El-baset[35] showed that using high concentration of amino acids as foliar sprays on both *Moringa oleifera*, and *Moringa ovalifolia* plants gave the highest amount of leaf vitamin C **content**.

Table (5): Means of Vitamin (C) content (27) of *Moringa oleifera* as affected by Bio-fertilizers, Amino acids, and Seaweed extracts and their combinations during 2020 and 2021 growing seasons.

Treatments	Vitamin C (27)						Mean
	Season2020			Season2021			
	1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut	
T ₀ Untreated	14.20	13.23	14.17	14.33	14.43	14.47	14.14
T ₁ Phosphorein	14.50	13.47	14.27	14.47	14.37	14.37	14.24
T ₂ Cerealine	14.53	13.03	14.27	14.57	14.33	14.60	14.22
T ₃ Seaweed 350mg/l	14.27	13.60	14.43	14.47	14.40	14.24	14.24
T ₄ Seaweed 350mg/l + Phosphorein 350mg/l	14.43	13.60	14.57	14.57	15.63	14.37	14.53
T ₅ Seaweed 350mg/l + Cerealine 350mg/l	14.37	14.10	14.50	14.57	14.20	14.43	14.36
T ₆ Seaweed 700mg/l	14.47	14.40	14.13	14.30	14.27	14.37	14.32
T ₇ Seaweed 700mg/l + Phosphorein 700mg/l	14.33	14.33	13.90	14.53	14.43	14.57	14.34
T ₈ Seaweed 700mg/l + Cerealine 700mg/l	14.53	14.27	13.63	13.63	14.33	14.50	14.15
T ₉ Amino 350mg/l	14.73	14.47	14.43	13.50	14.33	14.57	14.34
T ₁₀ Amino 350mg/l + Phosphorein	14.83	14.33	14.30	14.53	14.57	14.42	14.50
T ₁₁ Amino 350mg/l + Cerealine	15.33	14.67	14.43	14.50	14.30	14.47	14.62
T ₁₂ Amino 350mg/l + Seaweed 350mg/l	15.37	14.40	14.30	13.60	13.43	14.53	14.27
T ₁₃ Amino 350mg/l + Seaweed 350mg/l + Phosphorein	15.47	14.47	14.23	13.92	14.43	14.57	14.52
T ₁₄ Amino 350mg/l + Seaweed 350mg/l + Cerealine	15.53	14.47	14.30	13.77	14.13	14.67	14.48
T ₁₅ Amino 350mg/l + Seaweed 700mg/l	14.53	14.47	14.47	13.67	18.63	14.67	15.07
T ₁₆ Amino 350mg/l + Seaweed 700mg/l + Phosphorein	14.57	14.57	14.63	13.73	14.27	15.04	14.47
T ₁₇ Amino 350mg/l + Seaweed 700mg/l + Cerealine	14.53	14.27	14.90	13.73	14.80	15.20	14.57
T ₁₈ Amino acid 700mg/l	14.43	15.23	15.13	14.77	14.50	15.04	14.85
T ₁₉ Amino acid 700mg/l + Phosphorine	15.43	15.31	15.33	14.67	15.30	14.93	15.16
T ₂₀ Amino acid 700mg/l + Cerealine	15.57	14.70	15.47	14.53	15.33	14.85	15.08
T ₂₁ Amino acid 700mg/l + Seaweed extract 350mg/l	15.87	16.03	15.73	14.60	15.30	15.27	15.47
T ₂₂ Amino acid 700mg/l + Seaweed extract 350mg/l + Phosphorine	16.20	15.93	16.37	15.33	16.23	15.57	15.94
T ₂₃ Amino acid 700mg/l + Seaweed extract 350mg/l + Cerealine	16.23	17.40	17.03	16.33	16.30	15.89	16.53
T ₂₄ Amino acid 700mg/l + Seaweed extract 700mg/l	17.20	17.73	17.20	17.43	15.83	15.21	16.77
T ₂₅ Amino acid 700mg/l + Seaweed extract 700mg/l + Phosphorine	17.30	17.37	17.23	17.47	17.43	17.25	17.34
T ₂₆ Amino acid 700mg/l + Seaweed extract 700mg/l + Cerealine	17.33	17.57	17.80	17.73	17.27	17.22	17.49
L.S.D	0.26	0.66	0.42	0.32	0.38	0.41	

Total Carbohydrate ??

Concerning the impact of different investigated treatments on *M. oleifera* plants leaf total carbohydrates content, the data presented in **Table 6** indicates that leaf total carbohydrates content was significantly responded to the studied treatments. Furthermore, the plants that were sprayed with the amino acid and seaweed extract each at 700 mg/L with cerealine soil bio-fertilization (T₂₆) produced plants with the highest total carbohydrates content, with a mean value for both seasons of 34.66 ml/100 g as compared with the other investigated treatments. Followed by spraying with amino acid and seaweed extracts at 700 mg/L and Phosphorine bio-fertilizer (T₂₅) with a mean value for both seasons of 34.50 ml/100 g, which came in second rank. Such a trend was true during both seasons of study.

While that treatment with each individual, where cerealine (T₂) treatment alone gave a mean total carbohydrates content of 17.32 ml/100g, seaweed alone (T₆) gave 18.29 ml/100g, and amino acid alone (T₁₈) gave 25.32 ml/100 g. On the other way around, the untreated plants (control) (T₀) revealed the lowest value of leaf total carbohydrates of 17.06 ml/100 g for both seasons. Data showed that spraying with amino acid and seaweed extracts with soil bio-fertilization produced a better response than using each component separately.

These findings are in accordance with; Radwan [33] found that bio-fertilization enhanced the chemical composition of total carbohydrate percentage when applied to moringa plants. Also, Mazher[32] stated that they treated moringa plants with Phosphorine, Nitrobine, and Microbine biofertilizers and found that total carbohydrates was increased compared to the control. Likewise, Abd El-baset [35] showed that using high concentration of amino acids as foliar sprays on both *Moringa oleifera*, and *Moringa ovalifolia* plants gave the highest amount of leaf carbohydrate content. Also, Abdelkader [44] demonstrated that adding amino acids to mung beans and snap beans improved the total carbohydrates content. Moreover, Gajewski [45] found that adding fertilizer containing the algal extract *A. nodosum* and phosphorus improved the vitamin C content compared to the untreated cabbage.

Table (6): Means of Carbohydrate content of *Moringa oleifera* as affected by Bio-fertilizers, Amino acids, and Seaweed extracts and their combinations during 2020 and 2021 growing seasons.

Treatments	Carbohydrate (%DW)						Mean
	Season2020			Season2021			
	1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut	
T ₀ Untreated	17.33	17.33	16.67	16.30	17.30	17.40	17.06
T ₁ Phosphorein	18.07	17.33	16.37	16.50	16.67	16.37	16.89
T ₂ Cerealine	18.70	17.20	17.20	17.53	16.57	16.73	17.32
T ₃ Seaweed 350mg/l	17.33	17.10	17.23	17.70	17.30	16.83	17.25
T ₄ Seaweed 350mg/l + Phosphorein 350mg/l	19.37	16.97	18.03	18.63	17.83	17.50	18.06
T ₅ Seaweed 350mg/l + Cerealine 350mg/l	19.33	16.53	18.60	18.47	17.50	17.53	17.99
T ₆ Seaweed 700mg/l	18.50	17.20	18.50	18.37	18.17	19.00	18.29
T ₇ Seaweed 700mg/l + Phosphorein 700mg/l	19.87	17.40	18.23	18.33	16.37	19.33	18.26
T ₈ Seaweed 700mg/l + Cerealine 700mg/l	19.40	17.53	18.43	18.63	18.87	18.60	18.58
T ₉ Amino 350mg/l	30.40	17.37	19.13	18.30	18.10	18.41	20.29
T ₁₀ Amino 350mg/l + Phosphorein	20.37	18.43	19.57	20.17	19.43	19.40	19.56
T ₁₁ Amino 350mg/l + Cerealine	19.87	18.60	19.67	20.13	19.17	19.20	19.44
T ₁₂ Amino 350mg/l + Seaweed 350mg/l	20.40	19.13	20.53	24.57	19.12	19.57	20.55
T ₁₃ Amino 350mg/l + Seaweed 350mg/l + Phosphorein	22.00	16.43	20.50	24.40	20.41	20.4	20.69
T ₁₄ Amino 350mg/l + Seaweed 350mg/l + Cerealine	23.37	19.53	20.83	24.50	20.42	20.24	21.48
T ₁₅ Amino 350mg/l + Seaweed 700mg/l	20.37	20.13	21.43	29.93	20.20	20.50	22.09
T ₁₆ Amino 350mg/l + Seaweed 700mg/l + Phosphorein	21.53	21.33	22.97	30.43	22.63	21.00	23.32
T ₁₇ Amino 350mg/l + Seaweed 700mg/l + Cerealine	22.83	22.83	24.20	30.4	23.33	22.00	24.27
T ₁₈ Amino acid 700mg/l	23.57	23.80	24.13	31.60	24.77	24.07	25.32
T ₁₉ Amino acid 700mg/l + Phosphorein	24.47	25.67	28.00	31.60	26.43	26.10	27.05
T ₂₀ Amino acid 700mg/l + Cerealine	25.57	24.77	28.67	33.43	26.13	26.80	27.56
T ₂₁ Amino acid 700mg/l + Seaweed extract 350mg/l	25.67	23.70	30.87	30.03	39.47	29.17	29.82
T ₂₂ Amino acid 700mg/l + Seaweed extract 350mg/l + Phosphorein	27.30	24.67	32.37	34.43	30.30	30.07	29.86
T ₂₃ Amino acid 700mg/l + Seaweed extract 350mg/l + Cerealine	30.50	30.97	34.30	34.53	30.20	31.53	32.01
T ₂₄ Amino acid 700mg/l + Seaweed extract 700mg/l	31.37	33.07	34.53	35.53	31.20	31.60	32.88
T ₂₅ Amino acid 700mg/l + Seaweed extract 700mg/l + Phosphorein	32.50	33.43	35.13	35.50	35.57	34.84	34.50
T ₂₆ Amino acid 700mg/l + Seaweed extract 700mg/l + Cerealine	32.67	34.60	33.80	36.30	35.13	35.43	34.66
L.S.D	0.90	1.98	1.58	2.92	2.48	1.82	

Conclusion

Numerous variables that interact to either positively or negatively affect the chemical composition of the leaves are responsible for the substantial diversity in *M. oleifera*'s nutritional content. In the context of agronomic management, fertilization is the factor that most affects the chemical composition of the leaves. The results showed that bio-fertilization with phosphorine biofertilizers associated with amino acid and seaweed extracts each at 700 mg/L (T25) was the most effective treatment for enhancing and maximizing leaf nitrogen content. Also Herein, the most effective treatment that reflected the peak of leaf (phosphorus, potassium, calcium, vitamin C, and leaf total carbohydrates) content was observed with those plants that were soil bio-fertilized with cerealine bio-fertilizer with amino acid and seaweed extracts foliar spray each at 700 mg/L (T26) produced a better response than using each treatment separately. So, *M. oleifera* leaves that have a high nutritional content can be employed in human nutrition and protect against ailments caused by nutritional deficiencies. Thus, moringa leaves can serve as food to alleviate protein-energy malnutrition in food-insecure countries.

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