RESEARCH ARTICLE

Enhancing growth and nutrient uptake in boufegous date palm variety with seaweed extracts and AMF/PGPR combination in the field

Nogot Abdelaaziz[1*], Abdesalam Khardi[1], Hicham Aboumadane[2], Madiha Goutoutou[3], Jaiti Fatima[1]

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The utilization of Seaweed Extracts (SWE) and a combination of Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Rhizobacteria (PGPR) has received considerable attention in recent years due to their potential to ameliorate growth and nutrient uptake in diverse vegetable species. To promote sustainable agriculture in oasis ecosystems, particularly for the date palm that serves as a fundamental component of the economic and social development of oases, a study was conducted at a palm farm in Tamassint, Errachidia province, Morocco. The study aimed to evaluate the effects of Seaweed Extracts (SWE) and a combination of Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Rhizobacteria (PGPR) on the mineral uptake, physiological and morphological growth parameters of the boufegous date palm cultivar. Seven treatments were used, which included SWE: *Ascophyllum nodosum* at 1% and 2%, SWE: *Ecklonia maxima* at 1% and 2%, 10 g/palm and 20 g/palm of a combination of AMF/PGPR and a control group with no treatment. The results indicated that seaweed extracts enhanced growth rate parameters, nutrient uptake, chlorophyll content and fluorescence activity in both growing seasons, whereas the AMF+PGPR combination only improved all the measured parameters in the second year of the experiment, except for the number of leaves per palm where no effects were observed during both seasons.

Key Words: *Seaweed extracts; Arbuscular mycorrhizal fungi; Boufegous date palm cultivar; Growth; Nutrient uptake*

INTRODUCTION

In Morocco, the cultivation of date palms, with a diversity of 450 clones and varieties, is considered a pillar of social and economic development in oases, generating 3 million workdays, covering 59,600 hectares and yielding 102,000 tons per year [1]. This is steadily increasing year after year due to the establishment of new modern farms in the area. However, the lack of research on the biological management of date palm cultivation in these modern farms has led to excessive use of chemical inputs by farmers. This overreliance on chemical inputs has serious repercussions on the functioning of the oasis ecosystem, thereby threatening their biodiversity.

Use of biostimulants such as seaweed extracts, Arbuscular Mycorrhizal Fungi (AMF) and PGPR on various crops has shown their potential as organic alternatives to chemical inputs. SWE have been widely utilized in the form of foliar sprays or root drench applications to enhance the growth and productivity of fruit trees with positive effects on various plant parameters, including plant height, number of leaves per plant, root development, dry matter and nutrient uptake [2-5]. SWE also stimulate phytohormonal activity and natural processes responsible for shielding the plants against biotic and abiotic stresses, [6-9].

The current study evaluated the effectiveness of two seaweed extracts made from *Ecklonia maxima* and *Ascophyllum nodosum*, on plant growth and development. These seaweed extracts have positive effects on the growth parameters of various plant species. For instance, *Ecklonia maxima* increased nutrient content and absorption in the Zahdi date palm variety and displayed significant effects on the parameters of *Hordeum vulgare*, such as plant height, dry weight and leaf area [10,11]. *Ecklonia maxima* extract, which contains phytohormones, had significant growth-stimulating effects on *Spinacia oleracea* plants, leading to an improvement in yield, nutritional quality and cytokinin profiles [12]. *Ecklonia maxima*, contains cytokinin components such as zeatins, isopentenyladenine derivatives and other types, which are crucial in plant growth and development mechanism [13-16]. Similarly, *Ascophyllum nodosum* has positive effects on various plant parameters, including plant growth, resistance to abiotic stress and nutrient uptake, in several crops such as tomato, wheat and sweet pepper and promotes fruit retention and increases the size of the fruit [17-20]. Seaweed extracts derived from *Ascophyllum nodosum* modulate phytohormones and other molecular pathways, promoting plant growth and stress tolerance [21]. These extracts enhance plant defense gene transcription, antioxidant enzyme activity, phenolic content, cell resistance and plant growth and yield. In addition, the use of a combination of AMF and PGPR significantly improve various physiological and growth parameters, such as leaf water potential, electrical conductivity, stomatal conductance, photosynthetic pigments and efficiency [22,23].

The objective of this study was to assess the impact of applying commercial products containing seaweed extracts, namely Kelpak (SWE: *Ecklonia maxima*) and Algatop (SWE: *Ascophyllum nodosum*), as well as a product called Draks (consisting of a combination of AMF/PGPR, specifically *Glomus spp*., *Azotobacter spp*. and *Azospirillum spp*.) on the growth, development and nutrient uptake of two-year-old boufegous date palm cultivars during the 2021 and 2022 growing seasons. The effects of these biostimulants on two-year-old young plants because their formation at this stage is crucial for ensuring optimal productivity at maturity.

MATERIALS AND METHODS

Experimental design

A field experiment was conducted over the course of two seasons (2021-2022) in a privately-owned date palm orchard situated approximately 10 km from Errachidia city in the Draa Tafilalet region, Morocco (Figure 1). The study was conducting using 63 homogeneous boufegous palm variety cultivar (c.v) aged two years that were as uniform as possible in growth/vigor and unified number of leaves.

Boufegous palm trees were grown in sandy soil with an 8 m × 8 m spacing and irrigated using a drip system. Standard organic farming practices, which are commonly applied to date palms, were employed for the crop. The soil's chemical and physical characteristics were assessed meteorological conditions that prevailed during the study period were noted (Tables 1 and 2).

[1]*Department of Biology, FSTE, University of Moulay Ismail, Errachidia, Morocco;*[2]*Department of Agriculture, Agronomic Research Center, Tangier, Morocco;* [3]*Department of Agriculture, Regional Office for Agricultural Development, Tafilalet, Morocco*

Correspondence: *Nogot Abdelaaziz, Department of Biology, FSTE, University of Moulay Ismail, Errachidia, Morocco, E-mail: abdelaziznogot@gmail.com*

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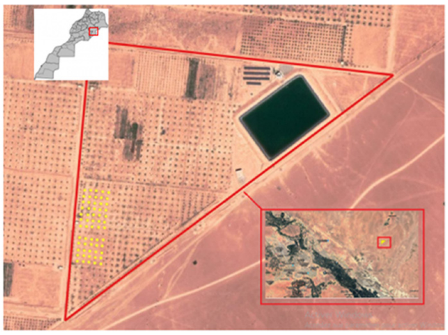

Figure 1) *Map indicating the localization of experimental trial in Errachidia, Morocco (X=-4,3294; Y=31,9589)*

TABLE 1

Chemical and physical contents of two soil horizons (H1=30 cm and H2=60 cm)

Soil characteristic	Horizon	Value	Soil characteristic	Horizon	Value
Sand	H1	58%	Available K_2O (mg.kg^{-1})	H1	336
	H2	64%		H2	425
Limon	H1	24%	Available P_2O_5 (mg.kg^{-1})	H1	4
	H2	20%		H2	7
Clay	H1	18%	Fer (mg.kg^{-1})	H1	1, 17
	H2	16%		H2	1, 27
CEC (meq/100 gr.soil)	H1	7, 2	Cu (mg.kg^{-1})	H1	0, 44
	H2	6, 4		H2	0, 46
%$CaCO_3$ total	H1	18, 4	B (mg.kg^{-1})	H1	0, 39
	H2	20, 9		H2	0, 41
%$CaCO_3$ actif	H1	8, 3	MgO (mg.kg^{-1})	H1	550
	H2	9, 4		H2	629
%OM	H1	0, 32	CaO (mg.kg^{-1})	H1	7764
	H2	0, 39		H2	7944
EC (ms/cm)	H1	0, 91	Zinc (mg.kg^{-1})	H1	0, 27
	H2	0, 77		H2	0, 77
C/N	H1	0	Na_2O (mg.kg^{-1})	H1	194
	H2	0		H2	216

Note: CEC: Cation Exchange Capacity; OM: Organic Matter; EC: Electrical Conductivity; C/N: Carbon to Nitrogen Ratio.

TABLE 2

Climatic data recorded in the site during trial period

Year	Month	ETo (mm)	Pluviometry (mm)	T°C		
				Min	Moy	Max
2021	May	7.1	5.2	16.2	24.25	32.3
	Jun	7.9	3.1	21.5	28.5	36.4
	Jul	8	0	23	28.9	40.3
	Aug	8.2	1.2	22.1	30.75	39.4
	Sept	6, 7	2, 6	18, 0	27, 5	35, 2
	Oct	4, 9	6, 6	10, 8	20, 2	28, 2
	Nov	3, 2	25, 4	3, 3	10, 7	18, 5
	Dec	2, 7	0, 0	0, 3	8, 4	18, 2
2022	Jan	2, 5	25, 8	-0, 7	6, 7	15, 3
	Feb	3,5	23, 2	3, 4	11, 1	18, 7
	Mar	4, 6	8, 8	5, 8	13, 5	20, 0
	Apr	6, 5	4, 0	10, 2	18, 3	25, 2
	May	7, 3	1, 0	14, 5	24, 1	30, 9
	Jun	8, 3	10, 2	19, 4	29, 5	36, 7
	Jul	8, 50	0, 40	23, 80	32, 70	38, 84
	Aug	7, 99	3, 40	24, 83	32, 83	38, 97
	Sept	6, 3	3, 0	19, 8	27, 4	33, 5

During both years of the experiment, a completely randomized block design was utilized with seven treatments and nine replicates, with each replicate consisting of one palm tree, for a total of 63 trees. The seven treatments used were Control, Kelpak (1% and 2%), Algatop (1% and 2%) and Draks (10 g and 20 g). The treatments were applied four times per year, at the beginning of May, June, July and August, by spraying seaweed extracts onto the leaves of the palm trees and applying a combination of AMF/PGPR products to the roots. The control palms were not treated (Figure 2 and Table 3).

Physiological and morphological parameters of date palm growth

Throughout the experimental period, monthly growth measurements were conducted four times per season to monitor the plant height (OA*), number of leaves per plant, number of leaflets per leaf and leaf length. The growth and growth rate were calculated using the following formula:

$$Growth = (M4 - M1)$$

$$Growth\,rate = {}^{(M4-M1)}\!/_{Trial\ day's\ number}$$

$$Growth\,rate/_{month(n+1)} = (M(n+1) - M(n)^{*})$$

$$Leaves\ number/_{plant} = Number\ of\ newly\ emerged\ leaves\ during\ the\ trial\ period$$

$$Leaves\ number/_{plant} = Main\ (Number\ of\ leaflets\ of\ 8\ newly\ emerged\ leaf)/_{plant}$$

OA*: The measurement from the soil line to the last fully expanded frond of a palm.

M (n)*: Measures of the month n.

Dry matter: Newly emerged leaves from each of the four cardinal directions were selected and leaflet samples were collected at the end of each season. For each tree 10 leaves were sampled. The dry matter content was determined by drying the samples at 105°C for 24 hours.

Leaf mineral contents: At the end of each growing season, a newly emerged leaf from each palm and collected a sample of 10 leaves per tree. These samples were washed, dried at 70°C until they reached a constant weight and then ground to determine the nutrient content using the methods described by Chapman et al., [24] for nitrogen, Jackson [25] for phosphorus and an atomic absorption spectrophotometer "Perkin Elmer 1100B" for potassium.

Chlorophyll content: The chlorophyll content in the leaflets was measured using a CCM-200 chlorophyll content meter. This was done for 8 leaves per plant, choosing them from different directions and positions. For each leaf, the chlorophyll content of 6 leaflets located in the upper middle and lower part of the leaf were measured.

Chlorophyll fluorescence: The chlorophyll fluorescence in the leaflets was measured using a fluorometer OS30p+on 12 leaves per plant from various directions and positions. For each leaf, the chlorophyll fluorescence of 12 leaflets located in the upper, middle and lower parts of the leaf was measured. The measurements were conducted at the end of each month during the trial period.

Statistical analysis: The obtained results underwent univariate statistical analyses, including one-way Analysis of Variance (ANOVA) followed by the Student-Newman-Keuls (SNK) test for comparison of means. All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) statistical software version 17.0 with a significance level of P<0.05.

RESULTS

Growth parameters

Palm height (OA): There were significant differences in growth rates from the first year of the trial by using Seaweed Extracts (SWE), There were significant differences between the combination of AMF/PGPR and the control only in the second season (2021). The highest growth rate of overall height (OA) was obtained during the second year of the trial with 2% of *Ecklonia maxima* (0.69 cm/day) and 2% of *Ascophyllum nodoseum* (0.64 cm/day), compared to the control group with a growth rate of 0.51 cm/day (Table 4).

During the heat and drought period (July and August) of the second year, there was a significant difference in the growth rate per month between the seaweed extract treatment and the other treatments (Figure 3).

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Déc
2021	Product Application					A1	A2	A3	A4				
	Measurement						M1	M2	M3	M4			
2022	Product Application					A5	A6	A7	A8				
	Measurement						M1	M2	M3	M4			

Figure 2) *Calendar of biostimulant application and measurement*

TABLE 3
Composition and characteristics of used biostimulant as given by the manufacturer

Product	Composition and characteristics
Kelpak®	34.26% seaweed extracts (*Ecklonia maxima*)
	1% total nitrogen
	5% phosphoric anhydride
	0.5% potassium oxide
Algatop®	25% seaweed extract (*Ascophyllum nodosum*)
	12.5% organic matter (algae)
	4% alginic acid
	4% water-soluble potassium oxide (K_2O)
	0.5% mannitol
Draks®	1% mycorrhizas (*Glomus spp.*)
	2.0*10^6 UFC/g *Azotobacter spp.*
	3.0*10^6 UFC/g *Azospirillum spp.*

TABLE 4

Effect of treatments on growth parameters of Majhoul date palm cultivar (c.v)

	T0	T1	T2	T3	T4	T5	T6
Season 2021							
Overall height (cm)	58.55 ± 0, 71^{a}	76.03 ± 1, 21^{c}	78.93 ± 0, 95^{d}	72.52 ± 0, 60^{b}	76.62 ± 0, 59^{c}	59.84 ± 1, 0^{a}	60.46 ± 0, 95^{a}
Leaves length (cm)	28.95 ± 0, 64^{a}	36.76 ± 0, 89^{b}	39.71 ± 0, 43^{c}	37.11 ± 0, 58^{b}	38.39 ± 0, 58^{b,c}	30.12 ± 0, 57^{a}	29.94 ± 0, 6^{a}
Leaves number	6.66 ± 0, 29^{a}	8.22 ± 0, 66^{b}	9.30 ± 0, 41^{c}	8.33 ± 0, 33^{b,c}	8.56 ± 0, 44^{c}	7.0 ± 0, 47^{a,b}	7.11 ± 0, 35^{a,b}
Leaflets/leaf	68.77 ± 1, 27^{a}	79.22 ± 0, 85^{b}	86.55 ± 1, 69^{c}	82.11 ± 2, 1^{b}	87.88 ± 1, 42^{c}	72.33 ± 1, 56^{a}	74.00^{a} ± 1, 53^{a}
Chlorophyll content	30.84 ± 2.01^{a}	48.51 ± 2.11^{b}	52.03 ± 1.86^{b}	48.13 ± 1.95^{b}	47.55 ± 2.01^{b}	34.4 ± 2.02^{a}	33.23 ± 1.78^{a}
Fluorescence (Fm/Fv)	0.61 ± 0, 01^{a}	0.68 ± 0, 01^{b}	0.69 ± 0, 01^{b}	0.69 ± 0, 01^{b}	0.70 ± 0, 01^{b}	0.64 ± 0, 02^{a}	0.64 ± 0, 01^{a}
Dry matter (g)	0.96 ± 0, 02^{a}	1.22 ± 0, 02^{b}	1.27 ± 0, 02^{c}	1.2 ± 0, 02^{b}	1.23 ± 0, 02^{b}	0.99 ± 0, 02^{a}	1.0 ± 0, 03^{a}
Season 2022							
	T0	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Overall height (cm)	62.39 ± 0, 80^{a}	76.99 ± 0, 78^{b,c}	85.36 ± 1, 44^{d}	76.69 ± 0, 7^{b,c}	79.5 ± 0, 95^{c}	71.37 ± 2, 28^{b}	74.4 ± 1,25^{b,c}
Leaves length (cm)	36.78 ± 0, 02^{a}	40.14 ± 0, 02^{b-d}	42.25 ± 0, 02^{d}	39.89 ± 0, 02^{b-d}	40.66 ± 0, 02^{c,d}	37.22 ± 0, 02^{a,b}	36.66 ± 0, 02^{a-c}
Leaves number	9.55 ± 0, 38^{a}	12.44 ± 0, 38^{c}	13.11 ± 0, 35^{c}	12.89 ± 0, 35^{c}	13.33 ± 0, 47^{c}	10.67 ± 0, 33^{a,b}	11.77 ± 0, 32^{b,c}
Leaflets/leaf	82.11 ± 2, 11^{a}	91.89 ± 1, 53^{b,c}	97.89 ± 1, 86^{c,d}	95.66 ± 1, 76^{c,d}	99.66 ± 1, 56^{d}	85.66 ± 2, 02^{a}	86.77 ± 2, 22^{a,b}
Chlorophyll content	36.82 ± 2.1^{a}	59.57 ± 2.03^{c}	66.7 ± 2.06^{d}	58.92 ± 1.59^{c}	60.87 ± 2.32^{c}	51.31 ± 0.96^{c}	54.76 ± 1.27^{c}
Fluorescent (Fm/Fv)	0.64 ± 0, 01^{a}	0.69 ± 0, 00^{c}	0.71 ± 0, 00^{c}	0.69 ± 0, 00^{c}	0.71 ± 0, 00^{c}	0.66 ± 0, 00^{b}	0.67 ± 0, 00^{b}
Dry matter (g)	1.01 ± 0, 01^{a}	1.34 ± 0, 03^{c}	1.46 ± 0, 05^{d}	1.26 ± 0, 03^{c}	1.36 ± 0, 03^{c}	1.08 ± 0, 03^{b}	1.16 ± 0, 03^{b}

Note: Average value ± standard error. Averages with the same letters in the same column are not significantly different at (p=0.05).

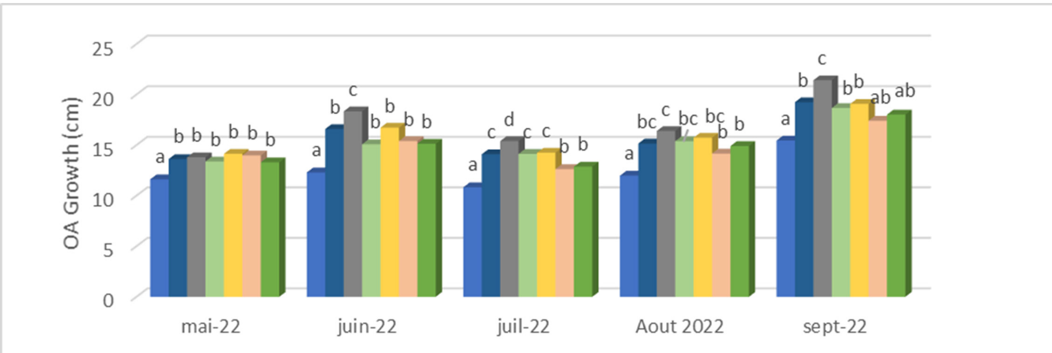

Figure 3) *Impact of biostimulant treatments on over all height per month;* **Note:** (■): *T0;* (■): *T1;* (■): *T2;* (■): *T3;* (■): *T4;* (■):*T5;* (■): *T6*

Leaf number and leaves length growth rate: During the two-season trial, plants treated with seaweed extracts showed a significant difference in both leaf length and leaf number when compared to the control treatment. The combination AMF/PGPR had a significant effect only on leaf length in the second year and did not have a significant effect on leaf number in either of the two seasons (Table 4).

In the second year, plants treated with 2% *Ecklonia maxima* and 2% *Ascophyllum nodosum* exhibited the highest growth rates in terms of leaves length (0.34 cm/day and 0.33 cm/day, respectively) and number of leaves per palm (13.11 and 13.33 leaves/palm, respectively) (Table 4).

Leaflets number and dry matter: The application of seaweed extracts significantly increased both parameters in both seasons. However, the combination only had a significant effect on the growth rate of dry matter in the second season (Table 4). Specifically, spraying with seaweed extracts at a concentration of 2% resulted in the highest dry matter content (T2=1.27 g and T4=1.23 g) and leaflets number (T2=97.89 leaflets/leaf and T4=99.66 leaflets/leaf).

Total chlorophyll content: Seaweed extracts had a significant effect on the total chlorophyll content of boufegous date palm in both seasons, with the injection of T2 at a concentration of 2% per palm resulting in the highest total chlorophyll content of 66.7 Chlorophyll Content Index (CCI), followed by T4 at 2% with 60.87 CCI in the second season. The combination of AMF and PGPR treatment significantly increased the chlorophyll content of date palm leaves during the second season when compared to the control (Table 4).

Chlorophyll fluorescent: There was a significant effect of seaweed extracts on the fluorescence parameter in both seasons. Specifically, the application of 2% Kelpak at a concentration of 2% and 2% Algatop resulted in the highest total value of the Fv/Fm ratio, with Fv/Fm=0.69 and Fv/Fm=0.70, respectively, compared to the control (Fv/Fm=0.61). Fluorescent measurements per month indicated a significant difference between the seaweed extract treatments and the other treatments during the period of heat and drought in July and August (Figure 4).

Nitrogen, a phosphorus and potassium content in date palm leaves: Application of seaweed extracts had a significant effect on the uptake of three essential minerals in the leaves of boufegous date palm variety, compared to the control treatments in both seasons (Table 5). The combination of AMF/PGPR had a significant effect on mineral uptake only in the second year. spraying with 2% *Ecklonia maxima* and 2% *Ascophyllium nodosum* extracts increased the nitrogen content of leaves by 2.17% and 2.15%, phosphorus content by 0.76% and 0.73% and potassium content by 1.19% and 1.38%, respectively. The control treatment resulted in the lowest levels of mineral uptake (Table 5).

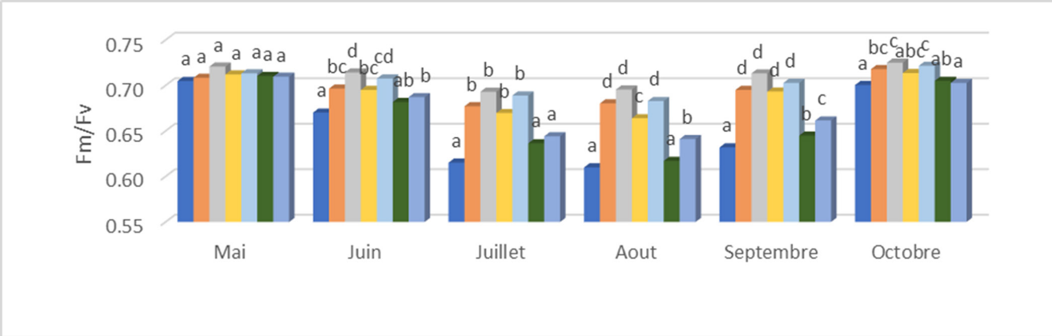

Figure 4) *The impact of biostimulant treatments on fluorescent parameter;* **Note:** (■): *T0;* (■): *T1;* (■): *T2;* (■): *T3;* (■): *T4;* (■):*T5;* (■): *T6*

TABLE 5

Phosphorus (P), Potassium (K) and Nitrogen (N) uptake in leaflets date palm

	Season 2021			Season 2022		
	N	P	K	N	P	K
T0	2.03 ± 0, 02^{a}	0.66 ± 0, 01^{a}	1.01 ± 0, 02^{a}	2.05 ± 0, 02^{a}	0.65 ± 0, 01^{a}	1.06 ± 0, 01^{a}
T1	2.15 ± 0, 01^{b}	0.72 ± 0, 01^{b}	1.16 ± 0, 02^{b}	2.20 ± 0, 01^{b}	0.77 ± 0, 01^{c}	1.22 ± 0, 04b,^{c}
T2	2.17 ± 0, 01^{b}	0.76 ± 0, 01^{c}	1.2 ± 0, 02^{b}	2.28 ± 0, 01^{c}	0.79 ± 0, 01^{c}	1.31 ± 0, 02c,^{d}
T3	2.13 ± 0, 01^{b}	0.71 ± 0, 01^{b}	1.16 ± 0, 01^{b}	2.20 ± 0, 01^{b}	0.75 ± 0, 01^{b,c}	1.28 ± 0, 03^{c,d}
T4	2.15 ± 0, 01^{b}	0.73 ± 0, 01^{b}	1.24 ± 0, 02^{c}	2.25^{b} ± 0, 01^{c}	0.76 ± 0, 01^{b,c}	1.33 ± 0, 02^{d}
T5	2.04 ± 0, 01^{a}	0.65 ± 0, 01^{a}	1.05 ± 0, 02^{a}	2.21 ± 0, 01^{b}	0.71 ± 0, 01^{b}	1.15 ± 0, 02^{b}
T6	2.07 ± 0, 01^{a}	0.66 ± 0, 01^{a}	1.05 ± 0, 01^{a}	2.25 ± 0, 01^{b,c}	0.75 ± 0, 01^{b,c}	1.18 ± 0, 03^{b}

Note: Average value ± standard error. Averages with the same letters in the same column are not significantly different at (p=0.05).

DISCUSSION

This study demonstrates that the application of seaweed extracts significantly influenced all growth rate parameters of boufegous date palm in both seasons, resulting in higher levels compared to the control treatment. These findings are consistent with previous studies reporting the positive effects of seaweed extract application on the growth and vigor of some others date palm cultivars such as Zahdi, Zaghloul, Bahree and Sukary, as well as other crops like tomato, *Ceropegia maculate* bedd and sweet pepper plants [26-30]. The increase in growth rate can be attributed to the improvements in various physiological activities, including nutrient uptake, chlorophyll content and chlorophyll fluorescence, recorded in the present study.

Combination of AMF/PGPR, required time to produce significant effects on the treated plants. No significant effects were observed during the first year, while significant improvements were noted in terms of leaf area, leaf length and leaf number during the second year.

This combination had no significant effect on the number of leaves per plant during the two years of the study. Establishing a beneficial symbiotic relationship between microorganisms and plants takes time. With at least four months for mycorrhizal fungi to develop in date palm. Young plants grown in desert soils with low organic matter may experience slower symbiotic development. Therefore, promoting the use of AMF and PGPR technology in the early stages of date palm cultivation, especially during the nursery phase, could prove to be more effective and advantageous than introducing it after the palms have been planted.

The efficacy of seaweed extracts treatments was more pronounced during periods of high-water demand and elevated temperature, from mid-May to September, compared to other treatments. different crops exposed to drought or heat stress have recorded comparable outcomes.

The levels of major essential elements Nitrogen (N), Potassium (K) and Phosphorus (P) were significantly enhanced by marine algae extracts in the first year, while the AMF/PGPR combination only showed significant effects on these elements in the second year of the experiment. These findings confirm those obtained in other studies treating other species with algae extracts [31-33].

CONCLUSION

Based on the findings of this study, the application of seaweed extracts led to an improvement in nutrient uptake and an increase in physiological and morphological growth parameters. The combination of AMF and PGPR showed significant effects on all parameters only in the second year of the study. Considering the cost of biostimulants products, seaweed extracts can be used as an immediate and occasional solution to address mineral deficiencies and promote growth and development of date palms during periods of abiotic stress. However, for a more sustainable solution, it is recommended to use AMF and PGPR biostimulants during normal farming practices and in nurseries to enhance growth and nutrient uptake since they are more affordable than seaweed extract biostimulants.

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