

Optimization of Quali-Quantitative Production of Date Palm CV. ‘Mejhoul’ in Moroccan Oases by Biostimulants Application

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Abstract

In Moroccan oases, the major challenge for modern date palm production is mainly focused on increasing yield and quality of fruits as well as sustainability of the arid climate zones. The aim of this study is to evaluate effect of two organic biostimulants on yield and quality of dates.

The experimental trial was conducted out, in a conventional farm of palm date (*Phoenix datylifera* L.) cv. ‘Mejhoul’ during 2016 season, under pedoclimatic conditions of Tinejdad, Errachidia region, Morocco. To this end, the application of two biostimulants was performed by spraying and repeated three different times after pollination (on late April, beginning-July and mid-August). Three variants were compared (Control, Protifert LMW 6.3 and Vitazyme®), the experimental design is completely random blocks with three repetitions and three trees per variant (a total of 27 palm trees). Measurements were made on yield and pomological traits of fruits. In addition, reducing sugars quantification was made by HPLC.

Results obtained showed that treatment affected significantly all of the studied parameters. Indeed, Protifert LMW 6.3 increased the yield per tree by 18 and 27% compared to Vitazyme and Control, respectively. As for fruit dimensions, Protifert spawned longer dates over than Vitazyme® and Control, whereas both biostimulants induced fruit of the same width but larger compared to Control. In addition, fruit weight

ranged from a maximum of 14.50 ± 0.13 g recorded by Protifert LMW 6.3 and a minimum of 10.32 ± 0.15 g observed in the Control. However, fruits of treated trees by Vitazyme® were richer in flesh (92.62%) compared to those obtained by untreated trees (90.78%). Glucose and fructose content engendered by Vitazyme were slightly decreased in comparison with Protifert and Control.

Keywords: Yield, quality, Biostimulants, date palm (*Phoenix datylifera* L.), 'Mejhoul', HPLC.

1. Introduction

In Morocco, especially in traditional oases characterized by an arid climate, the low yield and quality of the dates are considered as major problems of the date palm cultivation. In fact, the average yield of dates fruits was estimated at 2.16 T/ha in 2016 (FAOSTAT, 2018), this quantity of production remains weak in comparison with universal leaders countries such as Egypt (35.19 T/ha), Saudi Arabia (6.63 T/ha) and Iran (5.51 T/ha), this weakness is mainly due to unfavorable pedoclimatic conditions and inadequate hydro-mineral nutrition. Recently, technology of agricultural practices is performed, besides classic fertilizers; many efforts have been put into developing new set of fertilization systems, and also for organic farming with lower fertilizer inputs (Tarantino *et al.*, 2015). Several biostimulants have been developed and marketed in order to partially alleviate the adverse effects of climate change and abiotic stress on plant development while guaranteeing organic and sustainable production, moreover, their impact on the environment is marginal (Colla *et al.*, 2014; Tarantino *et al.*, 2015 et Brown et Saa, 2015).

In the scientific literature, there are several definitions of the word Biostimulant studied by many searchers as Kauffman *et al.* (2007), duJardin (2012), Calvo *et al.* (2014), Halpern *et al.* (2015), duJardin (2015). The following definition is proposed by duJardin (2012) and validated by European Biostimulant Industry Council (EBIC, 2012): plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. This definition considers the agricultural functions of biostimulants, not on the nature of their constituents or on their modes of actions (duJardin, 2015).

Generally, these products are derived from an incredibly diverse range of biological and inorganic materials including microbial fermentations of animal or plant feedstock, living microbial cultures, macro, and micro-alga, protein hydrolysate, humic, and fulvic substances, composts, manures, food, and industrial wastes prepared using widely divergent industrial manufacturing processes (Hamza et Suggars, 2001; Calvo *et al.*, 2014; Tarantino *et al.*, 2015).

Specifically, the categories of plant biostimulants that we review are: microbial inoculants, humic acids, fulvic acids, protein hydrolysates / amino acids and seaweed extracts. Consequently, it is illogical to assume that there is a single mode of action (Calvo *et al.*, 2014, Tarantino *et al.*, 2015).

Nowadays, the use of biostimulants is increasing in agriculture because this products promote the nutrient uptake, growth and development of the plants, improve quanti-qualitative yield in most cases. The mechanisms behind the physiological and biochemical effects of biostimulants are still unknown (Tarantino *et al.*, 2015). Nevertheless, Brown and Saa (2015) suggested that biostimulants benefit plant productivity by interacting with plant signaling processes thereby reducing negative plant response to stress. This hypothesis recognizes the wealth of recent research demonstrating that plant response to stress is regulated by signaling molecules that may be generated by the plant or its associated microbial populations (Marasco *et al.*, 2012; Bakker *et al.*, 2014; Vandenkoornhuyse *et al.*, 2015).

Besides the beneficial effect of amino acids on various crop plants has been identified and reported, including the assimilation efficiency of nutrients essentially iron and nitrogen (Cerdán *et al.*, 2009; Ertani *et al.*, 2009). In addition, this category of biostimulants acts on the process of plant tolerance to abiotic stress (Ertani *et al.*, 2013).

Some studies on annual plants had demonstrated the beneficial effect of plant biostimulants on growth, development and tolerance to abiotic stress (Disciglio *et al.*, 2014; Tarantino *et al.*, 2015). In Horticultural crops, Tarantino *et al.* (2015) reported that 'Canino' and 'Farbaly' apricot (*Prunus armeniaca* L.) cultivars were positively responded to foliar application of some commercial biostimulants containing amino acids humic and fulvic acids.

In our knowledge, in Egypt, some investigators (Abdel-Galeil *et al.*, 2017; Abdel-Galeil *et al.*, 2016; Alaa El-Din *et al.*, 2017) scored a positive effect of biostimulants on date palm crop (acclimatization stage, vegetative growth, yield and fruit quality). Although, these researches stay insufficient, the purpose of this present study was to elucidate the effect of two biostimulants products, in terms of the yield and quali-quantitative characteristics of fruits date cv. 'Mejhoul' in the Moroccan oases.

2. Materials and Methods

2.1. Plant Material

This study was conducted during the season 2016, in a private orchard situated at Tinjedad village, Errachidia region, South East of Morocco (Figure 1). A total of 27 healthy palms cv. 'Mejhoul', aged 15 years were selected for experimentation with uniform vigor as possible according the physical characteristics.

Figure 1: Map indicating the localization of experimental trial in Tinjedad village, Errachidia region, Morocco



2.2. Experimental Conditions

Palms are planted at a spacing of 6 x 6 meter with rows North-South oriented, grown in a loamy-sand soil (Table 1), the plot was irrigated by a surface irrigation system using drilling water, standard agricultural practices were applied to all palms and weeds were handelly controlled on the palms entourage during the trial. Mechanical and physico-chemical characteristics of the tested soil are shown in Table 1 (according to Wilde *et al.*, 1985).

Table 1: Soil analysis results

Characteristics of soil	Values
Sand [$2.0 > \phi < 0.02$ mm]	87.80 %
Loam [$0.02 > \phi < 0.002$ mm]	3.16 %
Clay [$\phi < 0.002$ mm]	9.03 %
Texture (FAO)	Loamy sand
PH (1 : 2.5 extract)	7.87
EC (1 : 2.5 extract)	2530 μ S
O.M %	0.685
Total CaCO ₃	14%
Active CaCO ₃	1.15%
Mineral N (Kjeldhal)	6.125%
P (Olsen method)	5 ppm
K (Ammonium acetate)	214 ppm

2.3. Experimental Design

Three variants Control, Protifert LMW 6.3 (4 mL.L⁻¹) and Vitazyme® (1.3 mL.L⁻¹) were compared according to a completely randomized block device with 3 replicates and three palms per experimental unit. The characteristics of the biostimulants are showed in Table 2.

Table 2: Composition and Characteristics of used biostimulants

Protifert LMW 6.3	Composition (% w/w) and characteristics	Vitazyme®	Composition and characteristics
Dry matter	58	Triancanol	0.17 mg/ml
Organic matter	40	brassinosteroids	0.03 mg/ml
Total Nitrogen	6.6	B1 Vitamin	0.45 mg/100 g
Organic Nitrogen	6.3	B2 Vitamin	0.03 mg/100 g
Total amino acids	40	B6 Vitamin	0.19 mg/100 g
Free amino acids	13	Glycosides and others	Unkown quantities
Organic Carbon	22		

The application of two biostimulants was carried out in three times with identical label doses (end of April, Beginning-July and mid-August (Figure 2)). Practically, this operation was made by spraying the solution on the leaves palm trees and soil perimeter, Control palms have not been treated. Pollen used for pollination was collected from the same male in order to avoid metaxenic effect.

Figure 2: Calendar of biostimulants application (treatments).

Treatement	Month											
	April		May		June		July		August		September	
	15	30	15	31	25	30	15	31	15	31	15	30
Control												
Protifert LMW 6.3		★					★		★			
Vitazyme®		★					★		★			

Fruit samples were harvested at Tmar stage (fruits maturity), then, subjected to economic yield quantification immediately. Pomological characterization and chemical analysis took place in the laboratory.

2.4. Yield per Tree Quantification

On each palm, all spathes were harvested at 'Tmar' stage and weighed by an electronic balance; values of treatment were expressed in Kg/palm tree.

2.5. Pomological Characterization

2.5.1. Fruit, Pulp and Seed Weights

Morphometric measurements were carried out on a composite sample containing 150 harvested from three palms of treatment devised in 50 fruits per palm tree. Indeed, the weight of the whole date, flesh and seed was measured using an analytical balance (Denver mark. Germany).

The quality ratios were measured according to the following formulas (Taouda *et al.*, 2014):

$$P/D \text{ ratio (\%)} = \frac{\text{Flesh weight}}{\text{Date weight}} \times 100$$

$$S/D \text{ ratio (\%)} = \frac{\text{Seed weight}}{\text{Date weight}} \times 100$$

2.6. Fruit Dimensions

Fruit length (cm) and fruit width (cm) were measured with a digital caliper (Mitutoyo CD -15GP. Mitutoyo Co., Japan).

2.6.1. Qualitative Evaluation

Qualitative evaluation of the results was carried out according to certain classification criteria as presented in Table 3 (Acourene *et al.*, 2001).

Table 3: Qualitative evaluation of dates according to the criteria of some physicochemical and biochemical parameters

Parameter	Criteria	Value	Qualitative evaluation
Fruit lenght	Reduced	<3.5 cm	Bad character
	Medium	3.5 – 4 cm	Acceptable
	Long	>4 cm	Good
Fruit weight	Law	<6 g	Bad character
	Medium	6 – 8 g	Acceptable
	High	>8 g	Good
Fleshweight	Law	<5 g	Bad character
	Medium	5 – 7 g	Acceptable
	High	>7 g	Good
Fruit diameter	Law	<1.5 cm	Bad character
	Medium	1.5 – 1.8 cm	Acceptable
	High	>1.8 cm	Good
Moisture	Veryweak	< 10 %	Bad character
	Law	10 – 24 %	Acceptable
	Medium	25 – 30 %	Good
	High	> 30 %	Bad character
pH	Acid pH	< 5.4	Bad character
	Between	5.4 – 5.8	Acceptable
	Superior	> 5.8	Good
Total sugars	Law	0.5	Bad character
	Medium	50 – 70 %	Acceptable
	High	> 70 %	Good

2.7. Determination of Individual Sugars by HPLC

2.7.1. Samples Preparation

Analysis of the individual sugar was carried out according to the modified method described by Kafkas et al. (2006). Lyophilized flesh fruit is crushed manually using a ceramic mortar. 250 mg is taken for each sample and was dissolved in 25 mL of aqueous ethanol 80% and sonicated for 15 minutes at 80°C, the solvent was filtered by Whitman filter paper using Buchner funnel. The extraction was repeated 3 times by adding 25 mL of ethanol 80%, the filtered extracts are combined and placed in the steam to remove the solvent, the residues are dissolved with 1 mL of deionized water and the pH was adjusted to 9-10 with diluted NaOH (0.1 M). A cartridge of 1 g/6 mL is preliminarily packaged with 6 ml of methanol and 6 ml of deionized water. Then, the recovered sample (1 mL) was eluted slowly through the cartridge and the sugars (neutral compounds) was carried out in solvents twice with 2 mL of deionized water (pH = 7).

Finally, the sugars eluted (4 mL) was diluted with filtered distilled water to a final volume of 100 mL. So, the sample was prepared for HPLC analysis.

2.7.2. HPLC Analysis Conditions

An HPLC system (Jasco LC-Net II/ADC, Japan) was used for determination of individual sugar content. The separation was carried out using a REZEX RHM monosaccharide H⁺ column with exclusion of ions (300 x 7.80 mm; Phenomenex), contained in an isothermal oven at an adjustable temperature.

The mobile phase consists only of filtered deionized water discharged into the system by a PU-2089 Plus quaternary gradient pump. The HPLC system is connected to an intelligent RI-2031-Plus detector. The flow rate and the injection capacity were adjusted, respectively, to 0.5 mL / min and 20 µL. Separation of sugars from organic acids was carried out by cartridges of 1g / 6 mL and Chrompure SAX type. The identified sugars were quantified based on peak areas related to two external standards consisting of a mixture of sucrose, maltose, glucose and fructose at concentrations 0.2 and 0.4% each. The baseline was made by a white consisting only of filtered distilled water. The areas of the peaks were determined by the ChromNav software and sugar content of each sample was calculated from the corresponding chromatogram, with respect to calibration curve. Results are expressed in g/100 g dry matter.

The calculation of sugar concentrations was carried out using methods described by several authors (Genna et al., 2008; Piga et al., 2008; Erosy et al., 2003a; Melgarejo et al., 2003; Merguez Bernardez et al., 2004), with certain modifications, as following formula:

$$C = (C1 \times \frac{A}{A1} \times \frac{V}{M}) \times 100$$

Where:

C: Sample concentration;

C1: Standard concentration;

A: Area of peak sample;

A1: Area of peak standard;

V: Volume of dilution water (100 mL);

M: sample weight (0.250 g).

2.7.3. Standard Samples

Pure samples (+) Glucose, D (-) Fructose, D (+) Sucrose and D (+) Maltose were used as standard.

2.8. Statistical Analysis

The Data collected were analyzed by SPSS program (Version 20.0, IBM USA). Student-Newman-Keulstest was performed at $P=0.05$ on each of the significant variables measured. Variability between samples in the tables was expressed as standard errors (SE) of the means.

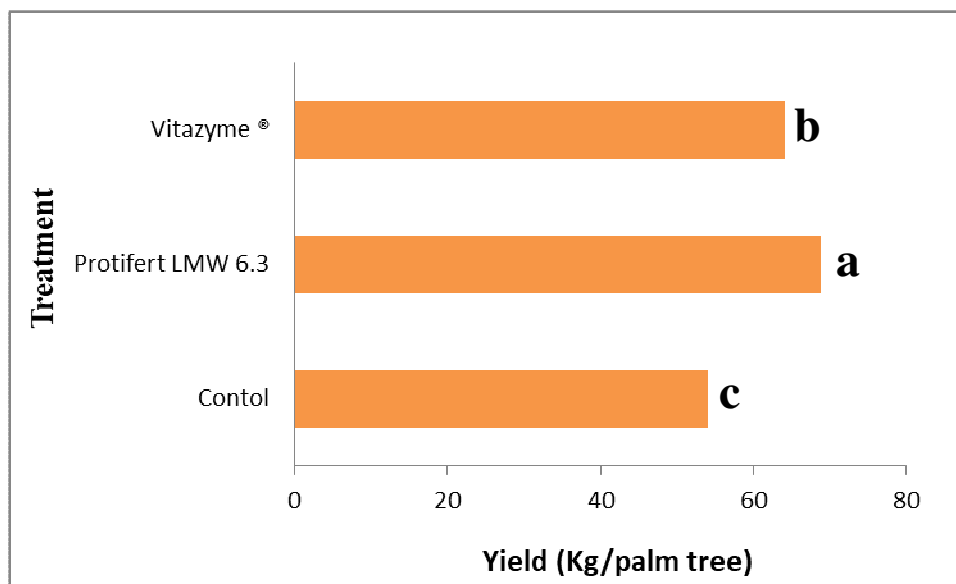
3. Results and Discussion

3.1. Yield of Date Fruits per Palm Tree

The yield values of date palm under two biostimulants treatment were reported in the Figure 3. Results show that significant differences were generated by both biostimulants. The highest economic yield was obtained by using Protifert LMW 6.3 (68.81 ± 0.47 Kg/palm tree) significantly different from Vitazyme® (64.07 ± 0.44 Kg/palm tree) and Control (54.10 ± 0.56 Kg/palm tree). Otherwise, compared to the Control, yield quantified of date palm was enhanced using Protifert LMW 6.3 and Vitazyme® by 27 % and 18.5 %, respectively. These biostimulants effects are considered positive for the quantitative production of date palm cv. 'Mejhouli'.

Economic yield measured were significantly influenced by biostimulants application. Obtained results are in agreement with published papers previously reported studies on biostimulant use in vegetable production (Russo, 1990; Maini, 2006; Vernieri, 2006; Parađiković, 2011 and Shehata et al., 2016). Except, dissimilar results were found in a previous study on apricot 'Canino' cultivar, total marketable fruit yield per tree were not affected by biostimulants treatment based on humic and fulvic acids, polysaccharide polymers and Nacetiltiazolidin- 4-carboxylic acid (Tarantino, 2017). This difference may be explained by the nature of exogenous biostimulants or the fruit specie treated.

Figure 3: Effect of Protifert LMW 6.3 and Vitazyme® on yield of date palm trees cv. 'Mejhouli'.



3.2. Pomological Parameters

All the studied morphometric characters of date fruits significantly increased on date palm treated by both biostimulants comparing with Control (Table 4).

As expected, fruit weights varied between a maximum of 14.51 ± 0.13 g recorded for Protifert LMW 6.3 and a minimum of 10.32 ± 0.15 g obtained by Control, both Biostimulants induced date fruits of the same width (2.32 ± 0.13 cm – 2.29 ± 0.12 cm, respectively), but wider than fruits of

Control trees (2.16 ± 0.13 cm). Besides, Protifert LMW 6.3 and Vitazyme® engendered longer fruits (3.96 ± 0.20 cm – 3.87 ± 0.18 cm, respectively) compared to Control (3.50 ± 0.22 cm). Fruits of palm trees treated with Vitazyme® were richer in pulp (92.62%) compared to those from untreated palms (90.78 %). Indeed, flesh ratio ranged between 90.78 ± 0.37 % recorded for Control and 92.62 ± 0.09 % for Vitazyme®.

According Qualitative evaluation of date fruits (Acourene et al., 2001), fruit weight and width was qualified as good and fruit length was acceptable for all treatments, for the fact that both biostimulants engendered maximum values. In this regard, Alaa El-Din et al. (2017), in Saudi Arabia, reported that treatment with 2% seaweed extract improved yield and the other fruit characteristics of date palm cv. 'Sukary'. Our results are in good harmony with those postulated by Parađiković et al. (2011) who declared that biostimulants based on amino acid increased weight of sweet yellow pepper (*Capsicum annuum* L.).

As regard commercial point of view, large dimensions of date fruits and high flesh ratio are considerably appreciated by consumers. Consequently, application of this biostimulants can be performed as a good production in put for promotion of date fruits marketing.

3.3. Individual Sugars

The important individual sugars profile and value in fruits date obtained for treatments as quantified using HPLC method are presented in Table 5, presented significant differences ($p < 0.05$) and standard error among fruits of different treatments. There existed significant differences between the final values of reducing sugars (glucose and fructose). Maltose and sucrose were not detected for all treatments. The obtained results showed clearly that Vitazyme® caused significant decrease in glucose (32.24 ± 0.91 g/100g DM) and fructose (29.06 ± 1.07 g/100g DM) contents as compared with control (35.01 ± 0.20 g/100g DM; 32.11 ± 0.25 g/100g DM). Indeed, maximal values were recorded for fruits treated by Protifert LMW 6.3 (35.02 ± 0.22 g/100g DM; 31.59 ± 0.27 g/100g DM).

The response of treated trees by Vitazyme® is in disagreement with a study related to apricot crop cv. 'Farbaly' (Tarantino et al., 2017); Authors found that the concentration of sugar (Brix°) in the fruits increased by all used biostimuants. But, the antioxidant capacity increased by some biostimulants and not affected by others. In addition, the phenolic content shows the highest values in the untreated control.

Table 4: Effect of Protifert LMW 6.3 and Vitazyme® on morphometric characteristics of date fruits cv 'Mejhoul'

Treatment	Fruit lenght (cm)	Fruit width (cm)	Fruit weight (g)	Flesh weight (g)	Seed weight (g)	Seed/Fruit Ratio (%)	Pulp/ Fruit Ratio (%)
Control	3.50 ± 0.22 c	2.16 ± 0.13 b	10.32 ± 0.15 c	9.42 ± 0.14 c	0.9 ± 0.01 b	9.22 ± 0.37 a	90.78 ± 0.37 b
Protifert LMW 6.3	3.96 ± 0.20 a	2.32 ± 0.13 a	14.50 ± 0.13 a	13.35 ± 0.16 a	1.16 ± 0.11 a	7.96 ± 0.70 ab	92.03 ± 0.70 ab
Vitazyme®	3.87 ± 0.18 b	2.29 ± 0.12 a	13.47 ± 0.15 b	12.49 ± 0.15 b	0.98 ± 0.01 ab	7.38 ± 0.09 b	92.62 ± 0.09 a

Average value \pm Standard error. Averages with the same letters in the same column are not significantly different at ($p = 0.05$).

Table 5: Effect of Protifert LMW 6.3 and Vitazyme® on individual sugars of date fruits cv.'Mejhoul'

Treatment	Glucose (g/100g DM)	Fructose (g/100g DM)	Total	Fructose/Glucose
Control	35.01 ± 0.20 a	32.11 ± 0.25 a	67.12 ± 0.01 a	0.91 ± 0.41 a
Protifert LMW 6.3	35.02 ± 0.22 a	31.59 ± 0.27 a	66.61 ± 0.01 a	0.90 ± 0.11 a
Vitazyme®	32.24 ± 0.91 b	29.06 ± 1.07 b	61.30 ± 0.01 b	0.90 ± 1.98 a

Average value \pm Standard error. Averages with the same letters in the same column are not significantly different at ($p = 0.05$).

DM: Dry Matter

Conclusion

The obtained results indicated that biostimulants application (Protifert LMW 6.3 (4 mL.L⁻¹) and Vitazyme® (1.3 mL.L⁻¹) enhanced markedly yield and major quali-quantitative characteristics of 'Mejhoul' date palm grown under Moroccan oasis conditions. However, the application of Protifert LMW 6.3 yielded the most interesting results in terms of yield and pomological attributes of fruits (fruit dimensions, fruit weight, flesh weight, seed weight, seed/fruit ratio, pulp/ fruit ratio).

These two biostimulants, whose effect could be explained by their involvement in the adaptation of the date palm to abiotic stress (hydric and saline) and the improvement of mineral assimilation. Hence, the studied biostimulants may be integrated in the management approach of date palm production under Moroccan oases conditions. Besides, Sustainable date palm production calls for the recruitment of ecological functionalities in case of protection and nutrition of oases system. In the future, further researches are required to better understand the role of biostimulants in mitigating the adverse effects of climate change on the date palm.

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