

Article



An Assessment of Seaweed Extracts: Innovation for Sustainable Agriculture

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Abstract: Plant growth regulators (PGRs) are described in the literature as having a significant role in securing crop management of modern agriculture in conditions of abiotic and biotic stressors. A joint field experiment was carried out to assess the role of seaweed-based extracts in pear trees and to test the "less for more" theory, which consists of getting more and better agricultural produce using fewer innovative inputs. The trials took place on two production seasons (from March till September 2018–2019) and the selected case study was on a pear orchard (*Pyrus communis* L. cv. Abate Fètel) in Emilia Romagna (Italy) by Fondazione Navarra and Timac Agro Italia S.p.A. Results demonstrate that, depending on the yearly climate conditions, it was possible to substantially reduce the primary nutrients by 35–46% and total fertilisation units applied by 13% and significantly improve quantitative and qualitative production indicators (average weight of fruits (5%) and total yield (19–55%)). Results also confirm a positive correlation between plant growth regulators and agronomic efficiency of pears which increased between five and nine times compared to the conventional nutrition programme. These outcomes constitute scientific evidence for decision making in farm management.

Keywords: pear trees; PGR; sustainable development; crop nutrition; fertiliser; Timac Agro Italia

1. Introduction

For decades, plant nutrition has been under scrutiny for concerns about negative externalities generated by the use of fertilisers in agriculture, which emerged in the late 1960s [1]. Since then, a clear correlation has been found between plant nutrition, the eutrophication of surface water, the accumulation of nitrate in water bodies and energy consumption. Even more recently, global studies have warned about unprecedented nitrate contamination of water [2], which is creating direct irreversible damage to natural ecosystems and human health [3]. Further, the most universal form of deteriorated water quality in the world in recent decades is freshwater eutrophication from phosphorus loss [4,5].

Looking at the glass half-full, the importance of fertilisers in agriculture has been extensively documented in the literature for over 150 years of research and experiments. The relevance of plant nutrition is fundamental for (i) normal growth and reproduction of crops [6], (ii) average crop yield increase [7] and (iii) improving soil fertility [8]. However, the fertilising rates have reached the optimum in the developed world, and the new direction is to reduce them. This has been one of the European Green Deal recommendations, for example, as expressed by the "farm to fork" strategy (The Farm to Fork (F2F) Strategy is at the heart of the European Green Deal set out in 2019 to make Europe the first climate-neutral continent by 2050. The strategy comprehensively addresses the challenges of sustainable food systems and recognises the inextricable links between healthy people, healthy societies and a healthy planet.) with a target of diminishing nutrient losses by at least 50% and reducing fertiliser use by at least 20% by 2030 [9].

The focus of scientific innovation is currently on crop biostimulants to activate natural plant processes, which, according to the documented literature, improve nutrient uptake and efficiency, crop quality and yield and build plant tolerance to abiotic and biotic stressors [10–12]. A statutory definition of biostimulants was provided in 2018 by the primary agricultural and food policy tool of the United States federal government (Farm Bill: https://www.congress.gov/115/bills/hr2/BILLS-115hr2enr.pdf). This definition is consistent with the one currently proposed by the European Bio-stimulant Industry Council (EBIC) (http://www.bio-stimulants.eu/) and in line with the definition under review by the European Union in the context of revising the existing EU regulation (EC) No. 2019/1009 relating to fertilisers (https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=OJ:L:2019:170:FULL&from=EN).

The definition sums up the scientific aspects raised in the literature and describes a plant biostimulant as "a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield".

Nevertheless, du Jardin [13] identified in a review study seven categories of biostimulants: (i) humic and fulvic acids, (ii) protein hydrolysates and other N-containing compounds, (iii) seaweed extracts and botanicals, (iv) chitosan and other biopolymers, (v) inorganic compounds, (vi) beneficial fungi and (vii) beneficial bacteria. This emerging field of research is very promising and represents one of the fundamental management aspects of agro-systems to reach sustainable agriculture that is more resilient to climate change and able to feed the increasing population [14].

Therefore, the literature still needs to explore different research aspects related to the biostimulant categories and their use in agriculture to answer evolving enquiries that arise with the technological advances in this field. Recently, algae have proved to contain natural active compounds with biostimulation and/or bioregulation effects [15], e.g., phytohormones, hormone-like substances, vitamins, antibiotics, amino acids, and primary, secondary and micro-nutrients.

Even though several forms of applying algal constituents have been reported in the literature, algal extracts from seaweed have proved to be the most efficient in terms of growth enhancement and stress tolerance [16–18]. Indeed, Mutale-joan et al. [16] have tested 15 different Crude Bio-Extracts (CBEs) obtained from acid hydrolysis of microalgae on tomato plant growth, chlorophyll content, nutrient uptake and metabolite profile. The authors have recorded positive effects on plant development, particularly, significant root and shoot length improvement and increased nutrients uptake. Further, Shukla et al. [17] have reviewed the ability of Ascophyllum Nodosum Extracts (ANE) to improve plant growth and agricultural productivity and have confirmed the plant growth promotion, the improvement of root/microbe interactions and nutrient use efficiency in plants and enhancement of plant tolerance to abiotic and/or biotic stresses. Finally, Michalak et al. [18] have successfully tested different seaweed extracts to enhance carotenoid and chlorophyll content in plant shoots and develop root thickness and above-ground biomass.

In this context, this paper proposes to explore the category of seaweed extracts produced by Timac Agro Italia, the Italian holding of the French multinational Groupe Roullier, a world leader in the field of plant nutrition, with the largest private research centre in Europe dedicated to plant physiology and nutrition and investing in these technologies. The selection of the trial crop also has significance, because pears are one of the major fruits of temperate climates, grown in almost all four corners of the world, reaching a total harvested area of 1.5 million hectares in 2018 and over 23.5 million tons of production according to FAOSTAT [19] (Figure 1). The tree belongs to two species: the common pear cultivated mainly in Europe, the Near East, America and Australia and known as the European pear (*Pyrus communis L.*) given its European descendants and the Nashi or Oriental pear (*Pyrus pyrifolia*) widely grown in Asia.



Area Harvested (% of total hectares)

Figure 1. Harvested area of pear (**top**) and total production (**bottom**) in the top 3 countries and the world.

2. Materials and Methods

The experiment was carried out in Emilia Romagna (Italy) at an Abate Fetel orchard for the relevance of this cultivar in Italy, which happens to be the main producer of pears in Europe [20], the third producer in the world in terms of area harvest and the second after China in terms of total production.

There are over 3000 identified pear cultivars worldwide [21], and in Italy, Abate Fetel (also known as Abbé Fetel) and 3 others (Conference, Beurrè Bosc, Doyenne du Comice) are the major cultivars commercially grown, providing more than 70% of the total annual production [22]. Further, the selection of Emilia Romagna has local importance, given that this region, in terms of fruit trees, is the first ranked in harvest area, production and the average size of farms (Table 1).

Region	Area Har	vested	Number o	of Farms	
	(ha)	(%)	(N)	(%)	
Emilia Romagna (EMR)	67,454.3	15.9	18,355	7.8	
Campania (CAM)	58,836.7	13.9	32,133	13.6	
Sicily (SIC)	54,295.5	12.8	36,055	15.3	
Piedmont (PIE)	43,673.3	10.3	20,168	8.5	
Lazio (LAZ)	36,318.8	8.6	15,323	6.5	
Sum of top 5 regions	260,578.5	61.4	122,034	51.7	
Other regions	163,725	38.6	114,206	48.3	
Total Italian fruit farms	424,303.5	100	236,240	100	
Average Italian fruit farm size		1.	8 ha		
verage fruit farm size in EMR		3.	7 ha		
Former elem	Area Har	vested	Number o	Number of Farms	
Farm size	(ha)	(%)	(N)	(%)	
Small (<10 ha)	222,270.4	52.4	201,324	85.2	
Medium (10–50 ha)	150,171.9	35.4	30,674	13.0	
Large (>50 ha)	51,861.5	12.2	4242	1.8	
Total Italian fruit farms	424,303.8	100	236,240	100	
	Source: Ist	at [23]			

Table 1. Top 5 regions in fruit production and farm size in Italy.

2.1. Case Study

The experiment took place at the experimental field of the Navarra Foundation, a reference of agricultural knowledge for the Navarra agricultural technical institute and farmers of the north-east of Italy, given its contribution to the development of the region's agri-food sector through research, experiments, innovation and knowledge transfer.

The experimental field has a total area of ≈ 2.5 ha, similar to the average size of fruit tree farms in the area, and is located in Ferrara (Table 2), characterised by a warm and temperate climate classified as Cfa by the Köppen-Geiger system. The historic precipitation and temperature measured at a weather station in Ferrara (Table 2) between 1961 and 1990 revealed a yearly average temperature of 13.1 °C and rainfall of around 689.5 mm [24], with considerable rain at high temperatures in the driest months (Figure 2).

The trials took place over two consecutive seasons in 2018–2019 on a V-shaped orchard system planted in 2005 using 3.8 m spacing between rows and variable in-row spacing of 0.5 m, with a tree density of 5263 trees per hectare. The orchard was evenly irrigated with a drip system without any variation between the rows and was covered with black anti-hail netting.

The soil structure is silty clay loam according to the classification system of the United States Department of Agriculture (USDA) and silt clay according to the International Soil Sciences Society (ISSS). The general composition of the soil is about 60% silt, 30% clay and 10% sand; it presents low compaction risk, high fertility indicators (organic matter 2.21%, C/N = 8.87) and a high content of available nutrients, given that the field is experimental with continuous trials carried out yearly. Soil tests were carried out before and during the experiment to guide the definitions of annual fertilisation programmes.

Table 2.	Geospatial	coordinates of	f experimental	l field and	weather	station of	reference

Location	Latitude	Longitude	Altitude
Experimental field	44.857 N	11.653 E	5 m
Weather station	44.861 N	11.656 E	4 m
	Source: [24,25].	



Figure 2. Historic average of precipitation and temperature in Ferrara (1961–1990) (after [24]).

The fertilisation programme was divided into 3 treatments: control, grown without any fertilisation; conventional treatment (CF), representing empirical nutritional treatment (primary, secondary and micro-nutrients and organic matter of animal or vegetable origin) conceived from the available products in the region (to simulate a conventional nutritional programme); and Timac Agro treatment (TIMAC), corresponding to a programme based on integrating conventional nutrients (primary, secondary and micro-nutrients and organic matter of animal or vegetable origin) with cutting-edge technologies created to reduce the environmental burden of fertilisers, increase the profit of farms and improve their well-being. The total fertilisation units per hectare for each treatment are reported in Table 3, which shows a great difference in the quantity of fertiliser applied in the 2 years. This difference is mainly due to the general climate conditions during the year, which considerably determines the quality and quantity of agricultural yield.

Type	Flement	2018 FU ha ⁻¹			2019 FU ha ⁻¹		
-) [-	Licificiti	CF	TIMAC	TIMAC/CF	CF	TIMAC	TIMAC/CF
	Nitrogen (N)	205.1	141.3	68.9%	174.9	120.7	69.0%
Primary Nutrionte	Phosphorus (P2O5)	184.4	79.0	42.8%	103.1	77.6	75.3%
Timary Numerus	Potassium (K ₂ O)	292.7	145.1	49.6%	246.0	140.8	57.2%
	Total Primary Nutrients	682.2	365.4	53.6%	524	339.1	64.7%
	Calcium (CaO)	42.5	46.4	109.2%	4.8	43.2	900.0%
Secondary	Magnesium (MgO)	21.6	24.1	111.6%	3.0	35.8	1193.3%
Nutrients	Sulphur (SO ₃)	109	200.2	183.7%	49.5	197.6	399.2%
	Total Secondary Nutrients	173.1	270.7	156.4%	57.3	276.6	482.7%
	Boron (B)	0.45	0.88	197.5%	0	1.45	_
	Copper (Cu)	0.43	0.06	13.9%	0	0.05	_
	Iron (Fe)	5.83	3.90	66.9%	2.25	1.50	66.7%
Micro-Nutrients	Manganese (Mn)	0.24	0	-	0.03	0.07	233.3%
	Molybdenum (Mo)	0.01	0.04	655.7%	0	0.30	-
	Zinc (Zn)	0.31	0.10	32.3%	0.02	0.11	550.0%
	Total Micro-Nutrients	7.26	4.98	68.6%	2.30	3.48	151.3%
OM	Total Organic Matter	43.8	44.4	101.3%	48.7	41.2	106.1%

Table 3. Fertilisation units (FU) applied per treatment and hectare in 2018 and 2019 seasons.

A supplement of complexed seaweed-based extracts was added to the TIMAC treatment (58 L ha⁻¹) in different growth stages and concentrations (Table 4) of three technologies: Fertiactyl[®], NMX[®], Seactiv[®]. These technologies are registered in the European Patent Office (EPO) under the numbers EP0609168, EP1147706 and EP0855375, respectively. The species involved in the extraction are *Lithothamnion corallioides*, *Lithothamnion glaciale*, *Lithothamnion tophiforme* and *Phymatolithon calcareum* and the general composition of the technologies are as follows:

Table 4. Quantity of seaweed-based technologies applied in Timac Agro treatment (TIMAC) treatment and corresponding growth stage.

Technology	Quantity (L ha ⁻¹)	Growth Stage
Fertiactyl®	8	
NMX®	3	Vegetative growth
Seactiv®	3	
NMX®	6	Emuit act
Seactiv®	6	Fruit set
Seactiv®	3	Post-harvest

Fertiactyl[®]: based on organic substrates acids whose fulvic and humic acids have been mobilized through the formation of soluble inorganic salts, phenolic and polyphenolic acids and zeatin;

NMX[®]: based on precursor, inhibitor and simulating enzymes (e.g., precursor compound of cyclic AMP; inhibitor compound of the enzymes of the Phosphodiesterases, stimulating compound of the enzymes of the Adenyl-Cyclase, etc.), mixed with mineral fertilisers and plant growth regulators (PGRs) (e.g., auxins, cytokinins, gibberellins, n-ethanolamines, polyamines, sugars, etc.);

Seactiv[®]: based on adenine derivatives to help, as foliar treatment agent, the migration and distribution within plants of nitrogenous nutrients and/or micro-nutrients.

The concentration in the different stages did not vary between 2018 and 2019.

2.2. Statistical Analysis

The experimental design was a randomised block design to minimise the effects of systematic errors. This design consisted of dividing the experimental block into 3 fertilisation treatments randomly selected within the block, with 2 replicates of 5 trees for each treatment. In total, 60 trees were used for data collection (all performed manually) and statistical analysis to determine whether mean scores differed significantly across the treatments. The measurements performed were divided into 3 pillars as follows:

Total harvest (t ha⁻¹); Average fruit weight (g);

Flower density $=$ FBT $=$ Number of Floral Buds per Tree	(1)
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$$Fruit density = NFT = Number of Fruits per Tree$$
(2)

Fruit set
$$=$$
 FS $=$ (NFT/FBT) \times 100 (3)

The collected field data were statistically examined, separately for each year, using analysis of variance (one-way ANOVA) with statistical probability ($p - value \leq 0.05$) and Tukey's honestly significant difference (HSD) test, which is a single-step multiple comparison procedure to find significantly different means. An excel spreadsheet has been used for this purpose.

The assumptions of both tests are essentially the same and they are three: normality (experimental errors of the data are normally distributed), homogeneity (equal variances between treatments) and independence (each sample is randomly selected and independent).

3. Results

Plants within a population often vary in the numbers of open flowers and fruits. The correlation between these two indicators is calculated by the fruit set, a ratio defined as the transition from flower to young fruit. These quantitative indicators in the development process of any plant are correlated to the rate of pollination [26] and determine the final yield quantities (or total harvest).

Field data for two consecutive years demonstrated an increase in all quantitative indicators under the TIMAC treatment compared to the conventional treatment and the control, which generated the highest harvest for TIMAC treatment. The literature has mentioned different abiotic and biotic stressors which could lead to flower drop in the early season [27]. In this experiment, the difference between the two years of trials is substantial and can be explained by the yearly climate variability in Italy. Indeed, 2019 was a dry winter with many clear-sky mornings generating diamond dust and leading to frost flowers, this generated a lower flower density trend in 2019 compared to 2018. Yet, TIMAC treatment has participated in avoiding further damage with higher flower density compared to the other treatment.

Even though the numerical difference is considerable, statistical significance is present only between the control and TIMAC treatments. Complete statistical results are listed in a final table (Table 5).

Indicator	Treatment	First Year, 2018			Second Year, 2019		
multutor	incutinent	Mean	Std Dev.	Variance	Mean	Std Dev.	Variance
Flower	Control	223.5 ^a	25.4	681.1	61.0 ^a	33.9	1211.0
Dongity	CF	219.4 ^a	28.7	865.8	53.1 ^a	28.3	844.6
Density	TIMAC	223.6 ^a	27.7	807.6	74.0 ^a	36.3	1384.2
	Control	38.2 ^a	10.5	115.4	11.1 ^a	6.1	39.8
Fruit Density	CF	42.4 ^{a,b}	18.1	343.2	13.9 ^a	9.4	93.7
-	TIMAC	50.4 ^b	17.1	308.4	25.1 ^b	11.4	136.2
	Control	17.4 ^a	5.3	29.4	25.2 ^a	22.9	551.6
Fruit Set	CF	20.1 ^a	10.2	109.5	30.1 ^a	17.7	328.1
	TIMAC	22.3 ^a	6.2	40.9	37.9 ^a	18.7	368.0
	Control	238.9 ^a	29.0	882.9	225.2 ^a	25.5	684.6
Fruit Weight	CF	237.1 ^a	31.8	1064.4	228.6 ^a	24.7	643.9
	TIMAC	242.7 ^a	24.5	633.8	230.6 ^a	30.1	952.4
Total Harvest	Control	47.5 ^a	13.1	180.7	13.5 ^a	8.1	69.7
	CF	51.6 ^{a,b}	19.8	413.5	16.1 ^a	9.9	104.0
	TIMAC	63.3 ^b	19.1	384.6	29.8 ^b	12.7	169.3

Table 5. Statistical results of selected indicators.

^{a,b} Letters indicate statistically significant means of different indicators.

However, high plant fertility would negatively affect the quality of the harvest, which, in conventional agriculture, would require flower removal intervention to boost the quality [28–30]. The role of plant regulators has been limited mainly to this growth stage of plants to control fruit set [31] and simultaneously boost the quantity and quality of yield [32].

While the average fruit weight of different pear cultivars can vary according to the genetic characteristics [33], within the same variety, fruit fresh weight is considered to be one of the most important quality indicators [34] determining the market value of the harvest. The effect of climate variability between 2018 and 2019, which initially generated a reduction in fruit density, led to a drop in the total harvest; however, the yield gap between the two treatments (CF vs. TIMAC) confirms the role of plant growth regulators (PGRs) in reducing crop response to abiotic stress. Further, fruit quality

did not undergo any variation. Figure 3 shows the qualitative and quantitative improvement of yield in the TIMAC treatment compared to the control and CF.



Figure 3. Improvement of fruit weight (top) and total harvest (bottom) under TIMAC treatment.

The outcomes of this experiment confirm the results of An et al. [31], showing a positive correlation between phytohormones application (auxin and ethylene) and fruit quantity and quality, and Bons and Kaur [32], who reviewed the effects of different PGRs on fruit set. The review included the impacts of 6-Benzyladenine (BA), 6-Benzylaminopurine (BAP), Gibberellic acid (GA3) and Naphthalene acetic acid (NAA).

Nutrient agronomic efficiency measures the technical performance of a crop and is calculated based on the yield difference between fertilised and unfertilised treatments, divided by the fertiliser units applied. Specifically, AE estimates productivity improvement gained by the use of nutrient input. Initially, it was used to evaluate nitrogen performance [35,36] and to improve the environmental and economic performance of agriculture, then it was extended to include the performance of phosphorus nutrition [37] to have a broader meaning and use agronomic efficiency correlated inputs for agro-system performance as an indicator of a transition to sustainable agriculture [38].

In this study, we calculated nitrogen, phosphorus and total nutrient efficiency for conventional fertilisation and TIMAC treatments (Table 6). The results show that the efficiency of the TIMAC treatment varied from 5.18 to 9.37 times higher than the conventional treatment (CF).

Year	Treatment	AE _(N)	$AE_{(P_2O_5)}$	AE _(Total)
	CF	20.1	22.3	4.8
2018	TIMAC	112.1	200.4	24.7
	TIMAC/CF	5.58	8.98	5.18
	CF	14.4	24.4	4.3
2019	TIMAC	134.9	209.8	26.3
	TIMAC/CF	9.37	8.59	6.10

Table 6. Nutrient agronomic efficiency of different treatments during experimental years.

4. Discussion

The results over two consecutive years of experiments show the role of PGRs in increasing crop tolerance to abiotic stress and improving physiological activities such as nutrient uptake and assimilation, reducing the total fertilisation units (FU) to around 13% (\approx 35.6 FU), which is an encouraging outcome towards the reduction of fertilisers according to the European Farm-to-Fork Strategy. Extrapolating this result to the total area of pears cultivated worldwide (\approx 1.4 million ha), this means saving a minimum of 49.8 million fertilisation units annually, without considering overfertilisation still practised in many countries around the world.

The results also reveal a substantial reduction in P_2O_5 use (over 45%), which is a significant result impacting the AE of phosphorus, and global efforts to reduce and/or substitute the use of phosphate rock, a mineral fundamental for food security, is expected to end in a short time [39,40]. The agronomic efficiency was higher in the second experimental, which presented higher abiotic stress due to climate conditions, affecting total yield. This confirms studies describing the important role of biostimulants as abiotic stress alleviators [41,42].

The field experiment confirmed the reviewed literature in Bons and Kaur [32], which assessed the positive correlation between plant growth regulators and the quality and quantity of harvests, as the TIMAC treatment improved both the quality and quantity of pears. Therefore, these results disproved the results of Dicenta et al. [43], which did not show a correlation between fruit set and total harvest.

There is not a consensus in the scientific community on one process to explain this because the mechanisms underlining seaweed extract-induced stimulation are still not completely revealed, though, several factors can be attributed to their activity. Literature has demonstrated that components within seaweed extracts may modulate innate pathways for the biosynthesis of phytohormones in plants [44]. It is also proved that marine bioactive substances (IPA extracts) enhance nutrient flux in [45] and increase endogenous antioxidant activity in plants [46]. Furthermore, the presence of phytohormones in seaweed extracts, as regulators of various cellular processes and responses, is presumably another factor that may have improved metabolic activity of crops [47] leading to better nutrition and yields.

Some questions that the research has raised and some future recommendations are mainly related to the importance of a balanced nutrition programme for sustainable management of crops. This can be defined by Liebig's law of the minimum, which is a fundamental principle in plant nutrition. This research partially demonstrates the importance of this law to the overall agronomic efficiency of crops (AE was not assessed in this study). Furthermore, it is recommended to follow the framework suggested by El Chami et al. [14], who proposed a methodology to reached sustainable agro-systems based on a life cycle study [48]; future studies will be intensified and address these questions, and will implement that methodology towards fulfilling the European Farm-to-Fork Strategy and the United Nations Sustainable Development Goals.

5. Conclusions

In conclusion, the experiment has confirmed the role of PGRs in reducing the total fertilisation units by 13% while improving the harvest between 19 and 55% and the quality of the fruit by about 5%. This improvement has positively affected the agronomic efficiency from five to nine times compared to the conventional nutrition programme. PGRs are, indeed, the precursors of a new agricultural

revolution which will transform the sector to sustainably face the present and future challenges of humanity. Thus, trials will continue to explore all aspects raised in the discussion to add applicable high-impact scientific evidence about the agronomic performance of PGRs.

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