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BIOESTIMULANTES NA PRODUÇÃO DE OLIVEIRAS EM DOM PEDRITO - RS

BIOSTIMULANTS IN OLIVE TREE PRODUCTION IN DOM PEDRITO - RS

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Resumo: A oliveira é conhecida por sua bienalidade, e sua polinização é cruzada. As espécies que possuem essa condição de fertilização contêm carga excelente de flores, porém índices baixos de fixação de frutos. Assim, o objetivo do trabalho foi avaliar a frutificação efetiva e consequentemente a produção, a partir de aplicações dos bioestimulantes Bio1 e Bio2, a dosagem utilizada foi de 150 mL para cada 100 litros de água. O experimento foi conduzido em dois anos onde foram realizados sete tratamentos: testemunha (sem aplicação); Bio1x3 - três aplicações de Bio1 na pré-florada, antese e 15 dias após a segunda; Bio1x2 - duas aplicações de Bio1 (pré-florada e antese); Bio1x1 - uma aplicação do Bio1 na antese; Bio2x3 - três aplicações do Bio2 pré-florada, antese e 15 dias após a segunda; Bio2x2 - duas aplicações (pré-florada e antese) e Bio2x1 - uma aplicação na antese. As variáveis avaliadas foram: fertilidade do grão-de-pólen, produção por planta, produtividade, massa média dos frutos e rendimento de azeite. O delineamento experimental utilizado foi em blocos casualizados, os dados foram submetidos às análises de variância (ANOVA) e as médias comparadas pelo teste de Tukey, utilizando-se o software WinStat. Os resultados experimentais apontam para vantagens do tratamento do bioestimulante Bio1 com diferenças de 40,50% e 27,03% da testemunha, enquanto o tratamento Bio1x2 foi 24,87%, 14,27% respectivamente superior à testemunha. Por fim o Bio1 aumentou a fertilidade do grão de pólen e consequentemente a produtividade da oliveira nos dois anos experimentais, também com a análise de custo-benefício positiva.
Palavras-chave: Aminoácidos. Fisiologia. *Olea europaea*.

Abstract: The olive tree is known for its biennial bearing, and its pollination is cross-pollinated. Species with this fertilization condition have an excellent flower load but low fruit set rates. Therefore, the aim of the study was to assess effective fruiting and consequently production through applications of the bio-stimulants Bio1 and Bio2, with a dosage of 150 mL per 100 liters of water. The experiment was conducted over two years with seven treatments: control (no application); Bio1x3 - three applications of Bio1 at pre-blossom, anthesis, and 15 days after

the second; Bio1x2 - two applications of Bio1 (pre-blossom and anthesis); Bio1x1 - one application of Bio1 at anthesis; Bio2x3 - three applications of Bio2 at pre-blossom, anthesis, and 15 days after the second; Bio2x2 - two applications (pre-blossom and anthesis); and Bio2x1 - one application at anthesis. Evaluated variables included pollen grain fertility, production per plant, productivity, average fruit weight, and olive oil yield. The experimental design used was randomized complete blocks, and data were subjected to analysis of variance (ANOVA) with means compared using Tukey's test, utilizing WinStat software. Experimental results indicate advantages of the Bio1 treatment with differences of 40.50% and 27.03% from the control, while the Bio1x2 treatment was 24.87% and 14.27%, respectively, superior to the control. Ultimately, Bio1 increased pollen grain fertility and consequently olive tree productivity over the two experimental years, with a positive cost-benefit analysis.
Keywords: Amino acids. Physiology. *Olea europaea*.

Introduction

The olive tree (*Olea europaea* L.) is one of the oldest cultivated plant species on record. It is a fruit-bearing plant with its fruit, known as olives, being a drupe belonging to the Oleaceae family. It originates from the region stretching from the southern Caucasus to the plains of Iran, with stronger indications that it originated in what is now Syria (CIVANTOS, 2017).

The land area planted with olive trees worldwide increased by 10% between 2014 and 2018, from 10 million hectares to 11 million hectares, according to data recently released by the International Olive Council (IOC, 2023). There were fluctuations from 2019 to 2021, and the projection until 2050 is that the global area will reach 15 million hectares.

The first olive tree specimens were introduced around 1800 in Brazil by European immigrants who settled in the Southern and Southeastern regions. However, initially, its introduction had only ornamental purposes in the southeast, with no significant cultivation at that time (OLIVEIRA, 2012). In Rio Grande do Sul, the first commercial cultivation took place around the 1940s in some municipalities of the state. Subsequently, in 1959, there was the introduction of some materials for research purposes, with some commercial areas, but significant advances in cultivated areas only occurred from the 2010s (JOÃO, 2022).

There are commercial olive tree plantations in the states of Rio Grande do Sul (in the southern half of the state, with examples in Encruzilhada, Canguçu, Caçapava do Sul, and Dom Pedrito), Minas Gerais (in Maria da Fé), and in the state of Santa Catarina, among other states with smaller proportions (AMBROSINI, 2022).

Complex studies involving chemical-histological techniques concluded that floral initiation begins in May (in Brazil), with the modification of floral buds resulting from the vernalization of buds (OLIVEIRA, 2012). The maturation of floral organs occurs up to 20 days before flowering, with the development of embryonic sacs and maturation of gametes. In the case of the olive tree, it is very rare for the pollen from one flower deposited on the stigma of the same flower to promote fertilization (CIVANTOS, 2017).

Olive tree flowering occurs in spring when temperatures reach about 15°C, resulting from the transfer of pollen grains from an anther to a receptive stigma at that moment, thus characterizing pollination (CIVANTOS, 2017). The olive tree flower exhibits self-incompatibility due to the delayed development of its pollen tube. When the pollen tube completes its passage through the stigma, there are no longer viable seminal primordia (RALLO; CUEVAS, 2008).

Floral buds initially swell, progressing phenologically to full flowering, followed by pollination and subsequently fruit setting. For this process to occur successfully, the average daily temperature needs to be approximately 8 to 20°C, and the relative humidity should be between 60 and 80% (COUTINHO *et al.*, 2009).

The olive tree has a considered biennial reproductive cycle, implying that two reproductive cycles are occurring simultaneously in this plant. This phenomenon leads to competition for products produced in photosynthesis (BUSCATTO *et al.*, 2017). According to Oliveira (2012), floral induction in the culture occurs seven to eight weeks after flower opening (during the hardening of the pit or endocarp of the fruits), a period when physiological changes in the meristem of a bud determine its differentiation. Gibberellins synthesized in the seeds of fruits during this period inhibit auxins in the growth tips of plants and consequently part of floral induction. This means that branches that have grown until this moment will produce in the next year. This fact allows, in years with many fruits producing gibberellins in the pit, the flowering in the following year to be lower.

Also, stemming from nutrition (in this case, nitrogen), the formation of essential amino acids in the genesis of hormones such as auxins, coping with water stress, pollen grain fertility, among other factors, can occur (GUPTA *et al.*, 2021). Therefore, this species, which has cross-pollination and, in its genotype, low pollen grain fertility, is a result of all these stressful environmental factors (SODINI *et al.*, 2023).

The particular functions of amino acids are well established, with specific amino acids being present. For example, glycine is involved in chlorophyll formation, glutamic acid is a key amino acid in the growth and functioning of meristems and fruiting, and proline and hydroxyproline and glutamic acid are responsible for pollen grain fertility and the consistency of cell walls (ALMADI *et al.*, 2020).

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As proven by Mosa (2021) in apple cultivation, the application of amino acids promotes an increase in the quality and quantity of production by positively influencing pollen grain germination, fruit setting, root system formation and strengthening, among other benefits. This occurs because plants have the ability to absorb amino acids through leaves and roots, representing a protein synthesis without energy expenditure (SILVA *et al*., 2021).

Regarding glutamic acid, there are studies indicating an improvement in pollen-pistil interaction and the orientation of pollen tubes in the Arabidopsis genus. Thus, a gradient of gamma-aminobutyric acid (GABA) concentration seems essential for the growth and orientation of pollen tubes (JIAO *et al.*, 2022).

Finally, GABA increases its action in sap when the plant is under stress, especially cold stress. For its production by the enzyme glutamate decarboxylase (GAD), Ca^{2+} is needed, along with the amino acid glutamic acid (ZHANG *et al.*, 2014).

Hou *et al.* (2023) consider the genetic factor to be relevant in fruit setting and pollen grain fertility. To ensure a good quantity of viable pollen and efficient fertilization of the oosphere, it is necessary that the stigmatic surface be compatible. Achieving this objective considerably increases olive tree fertilization with good fruit formation.

In the case of proline, a role as a free radical scavenger has also been reported (LIU, *et al.*, 2024). Besides participating in the secondary metabolism of Hydroxyproline, an amino acid responsible for the production of viable male gametes and genes forming this trait through the enzyme Hydroxyproline O-arabinosyl transferases (HPATs) (GOBI *et al.*, 2020).

In conclusion, this study aimed to increase crop productivity and reduce alternation through the use of biostimulants.

Material and methods

The experiment was conducted at “Rigo Vinhedos e Olivais”, located in the rural area of Dom Pedrito-RS-Brazil, where the topographical conditions expose the area to northeast-southwest solar radiation. The coordinates are latitude 31°08'46.71" S, longitude 54°11'53.80" W, and an altitude of 378 m. According to Köppen and Geiger (1928), the climate can be classified as Cfa. The soil is classified as an Argisoil in the Brazilian Soil Classification System, with moderate drainage.

Initially, the area was chosen, adopting a strategy of leaving a border row of plants to avoid the influence of other areas. Peripheral plants in the plot were also chosen for the same

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reason. The cultivar used in the experiment was Koroneiki, and the olive grove was established in 2010 using a polyconic training system with three main branches, spaced seven meters between rows and five meters between plants.

The experimental design employed a randomized block design with five repetitions of two plants, totaling 70 plant samples.

The study was conducted over two agricultural years, from August 2021 to the end of March 2022 (and repeated in the same months for the years 2022-2023). Seven treatments with two biostimulants were evaluated at three different times (pre-blossom, anthesis, and 15 days after anthesis). The control group received no biostimulant application. The organomineral biostimulants with a high content of essential free amino acids for plants were PhysioCrop Full (Fortgreen®) referred to as Bio1 and Radicel (Kimberlit®) referred to as Bio2, whose aminogram is described below in table 1.

Table 1 - Aminogram with free amino acid composition in (g. L^{-1}) and nutrient guarantee in (%) in biostimulants Bio1 (Physiocrop Full) and Bio2 (Radicel).

Description	Physiocrop Full (g . L^{-1})	Radicel (g . L^{-1})	Description	Physiocrop Full (%)	Radicel (%)
Aspartic acid	0,39	0,43	Nitrogen	9	3
Glutamic acid	10,99	5,49	Phosphor	2	1
Alanine	0,33	0,36	Potassium	1	1
Arginine	0,54	0,37	Sulfur	5	-
Phenylalanine	0,26	0,01	Boron	0,1	-
Glycine	0,61	0,90	Manganese	0,2	-
Isoleucine	0,33	0,89	Molybdenum	0,1	5
Lysine	13,15	1,53	Zinc	0,1	20
Methionine	1,13	3,45	Cobalto	-	0,5
Proline	3,54	0,14	Niquel	-	1,0
Serine	0,38	0,9	Total Carbon Organic	4,5	3
Tyrosine	0,46	1,67			
Threonine	1,22	2,30			
Tryptophan	0,88	0,30			
Valine	0,58	0,37			
Total	34,79	19,11			

Source: own authorship 2022.

The application regimen for Bio1x3 included three applications (x3) of Bio1: the first at pre-blossom on 14/09/21 and 06/09/22, the second at the beginning of flowering on 29/09/21 and 22/09/22, and the third at full flowering on 14/10/21 and 06/10/22 (15 days after the second). Bio1x2 had two applications (x2) of Bio1: the first at pre-blossom on 14/09/21 and 06/09/22, and the second at anthesis on 29/09/21 and 22/09/22. Bio1x1, the last treatment with Bio1, had only one application (x1) at the beginning of flowering on 29/09/21 and 22/09/22. Bio2x3 received three applications (x3) of Bio2: the first at pre-blossom on 14/09/21 and 06/09/22, the second at the beginning of flowering on 29/09/21 and 22/09/22, and the third at full flowering on 14/10/21 and 06/10/22 (15 days after the second). Bio2x2 had two applications (x2) of Bio2: the first at pre-blossom on 14/09/21 and 06/09/22, and the second at anthesis on 29/09/21 and 22/09/22. Bio2x1, the last treatment with Bio2, had only one application (x1) at the beginning of flowering on 29/09/21 and 22/09/22. The dosage for both biostimulants was 150 mL of commercial product per 100 L of solution, as recommended by the manufacturer.

Applications were made using a backpack sprayer, requiring an average of 0.650 liters of solution to cover each experimental area. The volume used was 13 liters for the application on 14/09/21 and 06/09/22, 19.6 liters for the second application on 29/09/21 and 22/09/22, and 6.5 liters for the third application on 14/10/21 and 06/10/22. These differences in application volumes were due to the number of treatments in each application.

Before the experiment installation, a soil analysis was conducted, and lime was applied for soil correction. Other macronutrients were also corrected, with only nitrogen being applied later as maintenance fertilization after fruit setting. Foliar applications of zinc and boron were made, with the latter also applied to the soil at a rate of 2 kg ha^{-1} due to its low phloem mobility.

Analyses Performed

The following analyses were conducted to assess which treatment had the greatest impact on olive orchard productivity and whether the cost-benefit ratio was significant for agriculture:

Pollen Grain Viability Test (%)

Conducted following the methodology adapted by Turner and Gillbanks (1974). The culture medium was prepared in an Erlenmeyer flask by dissolving sugar and agar in warm water. Afterward, 15% sucrose and 1% agar were added to 100 mL of distilled water.

The pollen grains were dispersed onto Petri dishes using a brush, covered with two sheets of previously moistened absorbent paper to simulate a humid chamber. The dishes were then incubated in a modified chamber at a controlled temperature of approximately 24°C for 48 hours.

The count of germinated and non-germinated pollen grains was performed under a tabletop microscope with a 10x10 eyepiece and objective. Those showing a pollen tube length equal to or greater than the pollen diameter were considered viable (KHALEGHI *et al.*, 2019). Pollen grains were randomly observed, and counts were recorded using a manual counter until reaching 100.

Total Fruit Mass (kg plant^{-1})

Measured using an analytical balance, the total mass per experimental unit was determined to correlate with the final production of each treatment. Fruit mass per plant and, consequently, the productivity per hectare for each treatment in kg ha^{-1} were also calculated.

Average Fruit Mass (g)

Determined from a random sample of 100 fruits, measured using an analytical balance.

Oil Yield (%)

Samples were processed using the compact machine model (RCM mini130) to analyze the oil yield for each treatment as an indicator of the increase in L.ha^{-1} and, consequently, the return in R$ per each invested real. The oil yield was calculated based on the total production for each treatment, considering that the extraction machine used was industrial and required a minimum quantity for optimal operation.

Cost Analysis

For this analysis, the methodology of Romanelli and Milan (2010) was employed. Initially, calculation parameters were outlined, followed by a descriptive report, and operational performance. Calculation parameters included:

-Calculation parameters:

a) Tractor Brand: New Holland model TL 60 E, new value R$100,000.00.

b) Sprayer: Jacto model Arbus 1500, new value R$136,000.00.

c) Fuel: Diesel oil R$6.20 per liter.

d) Tractor's average consumption: 3.8 liters per hour.

e) Reference salary: R$2,500.00.

f) Labor charges: R$1,250.00.

g) Accounting for 235 working hours per month.

h) Tractor's useful life: 8,000 hours.

i) Sprayer's useful life: 2,000 hours.

j) Scrap value: 20% of the new value.

k) Maintenance percentage: 75% for the tractor and 100% for the sprayer.

l) Average selling price per kilogram to the industry: R$3.00.

Calculations Report:

a) Tractor Depreciation = (New value - Scrap value) / Useful life.

b) Sprayer Depreciation = (New value - Scrap value) / Useful life.

c) Tractor Maintenance Cost = (75% of New value - Scrap value) / Useful life.

d) Sprayer Maintenance Cost = (100% of New value - Scrap value) / Useful life.

e) Interest on Capital invested in Tractor = (7% of New value - Scrap value) / Useful life.

f) Interest on Capital invested in Sprayer = (7% of New value - Scrap value) / Useful life.

g) Fuel Cost = (Market value * Average consumption per hour).

h) Labor Cost = (Salaries + Charges) / Working hours.

i) Operational speed in meters per hour.

j) Effective width in meters.

k) Time lost for adjusting the implement indexed at 0.8.

l) Field Time accounts for the estimated time lost without spraying in turns in the rows, indexed for this location at 0.75.

Spraying Operational Performance

Effective Performance = ((Effective Operational Speed x Effective Width) x Time lost x Field Time) /10000.

Data obtained for other variables were subjected to analysis of variance (ANOVA), and means were compared using the Tukey test ($p<0.05$), employing the statistical software WinStat (MACHADO; CONCEIÇÃO, 2002).

Results and discussion

Regarding the analysis of variance, the results for years 1 and 2 were significant at the 1% level for the variables pollen grain fertility, average production per plant (kg plant^{-1}), and average productivity (kg ha^{-1}). When means were compared using the Tukey test ($p<0.05$), the results described in Table 1 indicate that for the variables average production per plant (kg plant^{-1}) and average productivity (kg ha^{-1}), Bio1x3 outperformed the others in both years, differing by 40.50% and 27.03% from the control, while Bio1x2 was 24.87% and 14.27%, respectively, superior to the control. Although Bio2x3 had a lower increase, it also had a significant effect on the control, with a 9.09% increase in the first year. However, in the second year, it did not have a significant effect and, in fact, produced less than the control.

Table 2 - Pollen grain viability (%), Production (kg plant $^{-1}$), Productivity (kg ha $^{-1}$), average fruit mass (g), and Oil Yield (%), of "Koroneiki" olive trees treated with bio-stimulants (Bio1-Physiocrop full), (Bio2 -Radicel) in different treatments, Dom Pedrito, RS.

Treatments	Pollen grain viability(%)	Production (kg planta $^{-1}$)	Productivity (kg ha^{-1})	Average Fruit Mass (g)	Oil Yield (%)
			2022		
Control*	46.50c**	19.58d	5.580.300d	124.50n/s	13n/s
Bio1^{x3}	54.33aª	27.51ª	7.840.350ª	123.36n/s	13n/s
Bio1^{x2}	51.66b	24.45b	6.968.250b	118.58n/s	13n/s
Bio1^{x1}	50.33c	20.35d	5.799.750d	125.94n/s	13n/s
Bio2^{x3}	46.80c	21.36c	6.087.600c	119.92n/s	13n/s
Bio2^{x2}	47.40c	21.10c	6.013.500c	121.23n/s	13n/s
Bio2^{x1}	47.66c	19.73d	5.623.050d	122.45n/s	13n/s
CV(%)	5.55	33.30	33.30	4.65	-
			2023		
Control	47.10c	19.61c	5.588.85c	99.45n/s	13.5n/s
Bio1^{x3}	55.33ª	24.93ª	7.100.05ª	100.16n/s	13.5n/s
Bio1^{x2}	52.63b	22.41b	6.386.85b	98.87n/s	13.5n/s
Bio1^{x1}	49.83c	19.93c	5.680.05c	96.91n/s	13.5n/s
Bio2^{x3}	46.95c	19.87c	5.662.95c	101.56n/s	13.5n/s
Bio2^{x2}	47.20c	19.38c	5.523.30c	99.92n/s	13.5n/s
Bio2^{x1}	48.16c	18.99c	5.412.15c	101.15n/s	13.5n/s
CV(%)	6.04	9.59	9.59	1.43	-

*Control – no application, Bio1x3 three applications (x3) of Bio1: pre-flowering on 09/14/21 and 09/06/22, beginning of flowering on 09/29/21 and 09/22/22, and full flowering on 10/14/21 and 10/06/22. Bio1x2 two applications (x2) of Bio1: pre-flowering on 09/14/21 and 09/06/22, anthesis on 09/29/21 and 09/22/22. Bio1x1, application (x1) at the beginning of flowering on 09/29/21 and 09/22/22. Bio2x3 three applications (x3) of Bio2:

pre-flowering on 09/14/21 and 09/06/22, beginning of flowering on 09/29/21 and 09/22/22, and full flowering on 10/14/21 and 10/06/22. Bio2x2 two applications (x2) of Bio2: pre-flowering on 09/14/21 and 09/06/22, and anthesis on 09/29/21 and 09/22/22. Bio2x1, the last treatment with Bio2, had only one application (x1) at the beginning of flowering on 09/29/21 and 09/22/22; **Means followed by different letters in the columns differ statistically by the Tukey test at a significance level of 5%.
Source: own authorship 2023.

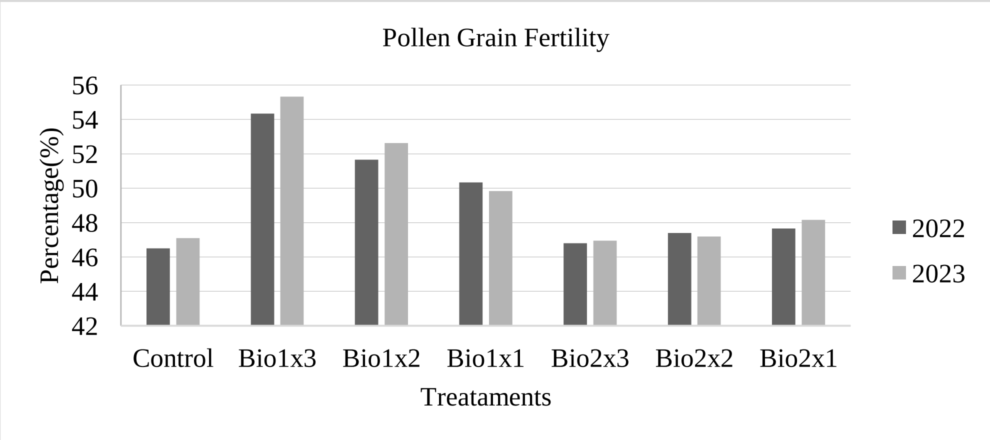
When comparing the bioestimulants, Bio1x3 showed significant differences compared to Bio2, with a difference of 28.79% and 25.37%, respectively, when comparing the best treatment of Bio1x3 to the best result of Bio2x3.

This result is due to Bio1 containing a higher concentration of the amino acids glutamic acid and proline, both of which act on the fertility of the pollen grain, consequently increasing the effective fruiting and productivity of the olive grove.

Starting with the variable pollen grain fertility, there was a significant difference at a 5% level by the Tukey test even with adjusted results, where Bio1x3 with three applications of the bioestimulant showed the best results (Figure 1).

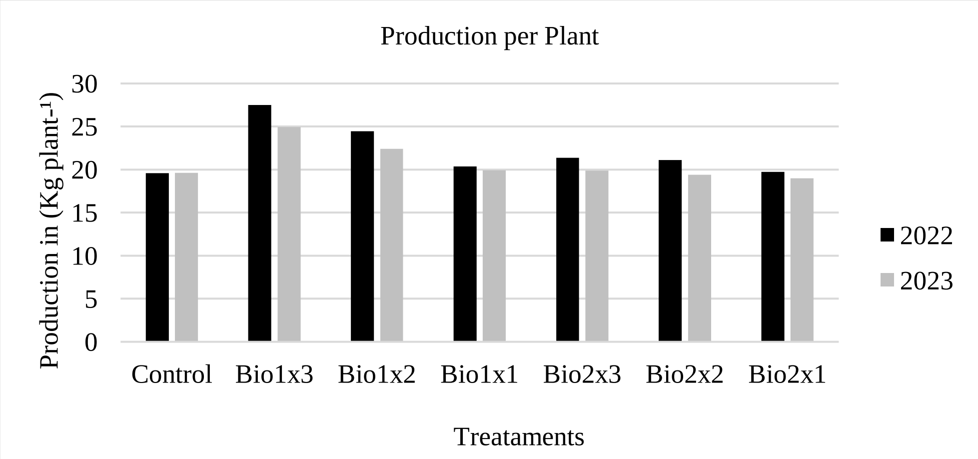
Figure 2 shows the variable production per plant, where there is a superiority of the Bio1x3 treatment, resulting in an increase of 40.50% and 27.03%, respectively, in production compared to the control.

Figure 1. Pollen Grain Fertility (%), under different treatments of two bio-stimulants, at distinct times in year 1 and 2, corresponding to the harvests of 2022 and 2023.



Source: own authorship 2023.

Figure 2. Production per plant (kg Plant-1), under different treatments of two bio-stimulants, at distinct times in year 1 and 2, corresponding to the harvests of 2022 and 2023.



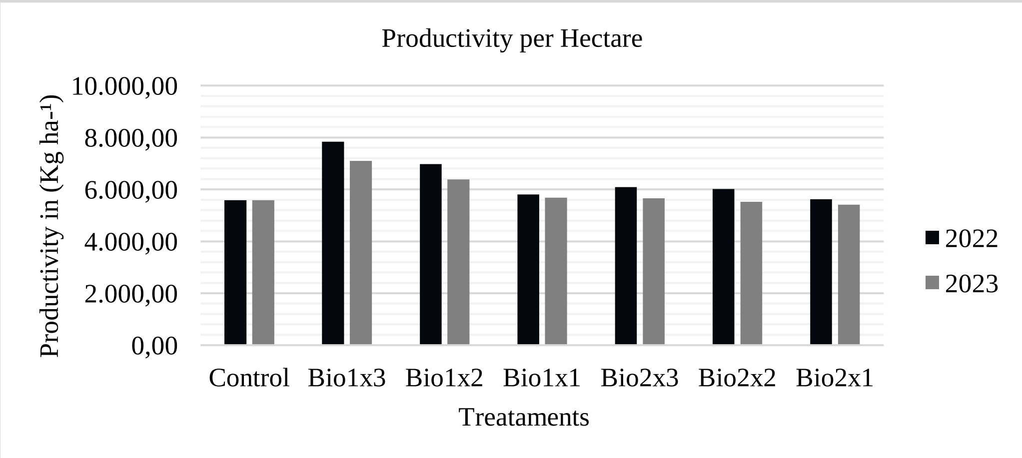
Source: own authorship 2023.

For the hectare productivity variable, a simulation was performed based on the average productivity per plant; therefore, the illustration in Figure 3 below remains identical.

The difference in average production between the two best treatments, Bio1x3 and Bio1x2, was 872,100 kg ha^{-1} and 713,200 kg ha^{-1}, respectively. The variables of mass of 100 fruits and oil yield did not show a significant difference, accepting the null hypothesis (H_0).

The oil yield was 13% and 13.5%, respectively, for all treatments, but the lack of reduction in the more productive treatments already indicates significance in the benefit of using the bio-stimulant.

Figure 3. Hectare productivity (kg ha^{-1}) under various treatments of two biostimulants at different time periods.



Source: own authorship 2023.

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To conduct the cost-benefit analysis, also known as opportunity cost, calculations were simulated in Tables 3 and 4 below. For this purpose, a commercial application scenario was established based on the optimal outcome from the evaluated treatments. A higher spray volume was considered due to tractor-mounted application, resulting in an expanded interval of treated plants. It was assumed that the concentration of the biostimulant would remain unchanged with a spray volume of approximately 350 L ha^{-1}, representing the typical scenario in a commercial olive orchard using empty cone nozzles with a fine droplet pattern. Combining three applications would result in a total volume of 1050 L ha^{-1}, incurring costs of R$ 35.00L^{-1} for Bio1 and R$ 210.00L^{-1} for biostimulant Bio2. Approximately 1,575 L of biostimulants would be expended over the course of these three applications.

Table 3 - Operational Cost Analysis.

Operacional costs	R$ $hour^{-1}$	hour ha^{-1}	R$ hectare $^{-1}$
Tractor Depreciation	10.00	0.396	3.96
Sprayer Depreciation	54.40	0.396	21.54
Tractor Maintenance	7.50	0.396	2.97
Sprayer Maintenance	54.40	0.396	21.54
Interest on Capital invested in Tractor	0.70	0.396	0.28
Interest on Capital invested in Sprayer	38.08	0.396	15.08
Fuel	23.56	0.396	9.33
Labor	15.95	0.396	6.31
Total	204.59	0.396	81.01

Source: own authorship 2022.

Table 4 -Economic response to the biostimulants Physiocrop Full (Bio1) and Radicel (Bio2) in the evaluated years 2021 and 2022.

	Operational cost of one application (R$ hectare $^{-1}$)	Biostimulant Cost (R$ hectare $^{-1}$)	Total cost per hectare* (R$ hectare $^{-1}$)	Production Variation Relative to Control** (R$ hectare $^{-1}$)	Economic Response*** (R$ $^{-1}$)
Bio1-2021	81.01	18.37	136.12	6.780.15	49.81
Bio1-2022	81.01	18.37	136.12	4.533.60	33.30
Bio2-2021	81.01	110.25	411.76	1.521.90	3.69
Bio2-2022	81.01	110.25	411.76	0.222.30	0.53

*(Operational Cost + (Biostimulant Cost x number of applications)) = Bio1 (Physiocrop Full) (81.01 + (18.37 * 3); Bio2 (Radicel) (81.01 + (110.25 * 3)). **Obtained Production (+ or -) kg x Product Price: Bio1 = 2260.05 kg X \$3.00 kg^{-1}; Bio2 = 507.30 kg X \$3.00 kg^{-1}. *** = Increase or decrease in R$ / production cost: Bio1 = \$6,780.15 / \$136.12 R$ $^{-1}$; Bio2 = \$1,521.90 / \$411.76 R$ $^{-1}$. Economic response variations between years were due to production fluctuations, rendering one of the biostimulants economically unviable in the second year.
Source: own authorship 2022.

Numerous articles in the field extensively discuss the role of amino acids in plant abiotic stress responses, conforme citado pelos autores Lee *et al.*, (2021), Liu *et al*, (2024), Mosa *et al.,2021*, Rallo *et al*, (2020). However, the specific function of amino acids in increasing pollen grain fertility was first tested by, attributing hydroxyproline (an amino acid derived from proline) as the key amino acid responsible for viable male gamete production and the genetic mechanisms regulating this trait through Hydroxyproline O-arabinosyl transferases (HPATs) enzymatic activity.

According to Khattab and Sidhu (2017), the maturation of floral organs in crops is dependent on the utilization of nutrients such as Boron (B) and Calcium (Ca), associated with amino acids during flowering. This combination can induce maturation up to 10 days before flowering, facilitating embryonic sac development and gamete maturation. This aligns with the experimental results, as treatments Bio1x3, Bio1x2, and Bio2x3 (pre-flowering application) yielded the most statistically significant results. It can be considered that bioestimulant applications after this phenological stage did not show an effect due to complete organ maturation, consequently not enhancing fruit set.

Basile *et al.* (2020) obtained similar results in blueberry cultivation in Portugal, attributing increased fruit set efficiency to bioestimulant application. The application during early bud break and pre-flowering also led to increased productivity in the treated area, with the most successful treatment applied during pre-flowering. These authors attributed this benefit to increased enzymatic activity of key enzymes, facilitated by the combination of amino acids, especially nitrate reductase and glutamine synthetase, as observed in this experiment. This is possible because amino acids have significant permeability through the cuticle via spray application and possess a quantity of free N and OM (Organic Matter), thereby enhancing the foliar absorption efficiency of enzymes (nitrate reductase and glutamine synthetase).

Lee *et al.* (2021) elucidated the action of the amino acid glutamic acid, causing an increase in photosynthetic efficiency and antioxidant enzyme activity. This amino acid, along with Ca^{2+}, in the formation of the enzyme glutamate decarboxylase (GAD), aids in the synthesis of GABA (gamma-aminobutyric acid), thus enhancing pistil orientation.

Conclusion

Based on the obtained results, it is feasible to assert that, under the experimental conditions during these study years, the commercial bioestimulant Bio1(Physiocrop full) demonstrated significance in both cycles by enhancing crop productivity. The viability of its application was notably positive, surpassing that of other alternatives.

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