DOI: 10.5937/ISAE25114S

ISAE 2025 - Book of Abstracts
The 7th International Symposium on Agricultural Engineering

THE INFLUENCE OF BIOSTIMULATORS AND BIOPROTECTORS ON THE QUALITY AND HEALTH STATUS OF GRAPES OF THE ŽILAVKA VARIETY

Amar Stranjak[1], Dženita Alibegić[2], Vedrana Komlen[1*]

[1]University „Džemal Bijedić“, Agromediterranean faculty, University Campus, 88 104, Mostar, Bosnia and Herzegovina
[2]University „Džemal Bijedić“, Study of Pharmacy, Sjeverni logor, 88 104, Mostar, Bosnia and Herzegovina

*vedrana.komlen@unmo.ba

Abstract. Unfavorable agro-ecological circumstances for grape agriculture have emerged in recent years as a result of climate changes, such as high air temperatures and a lack of precipitation. To counteract the negative repercussions, biostimulants are indicated for usage in plantations that have experienced stressful events such as drought, frost, nutrient deficiency, and plant disease. Furthermore, the usage of silicon-based preparations alleviates stress caused by insects, infections, and climate change. An experiment was conducted on Žilavka vines at a family farm in Kočina, near Mostar, during the 2025 crop year. Seven treatments were used to assess the impact of various preparations. To assess the influence of different kinds on mechanical composition, the weight of bunches, petioles, and berries were examined, as well as the sugar and acid content of grapes. Plasmopara viticola was also present this year, along with Lobesia botrana; both species caused serious damages. Every day, the vineyard's air temperature and precipitation were measured. Statistical data analysis using the ANOVA test revealed substantial differences between the variants, whereas the LSD test allowed for accurate comparisons of individual variants. The results demonstrate that Variant 5 significantly increased the weight of the bunches compared to the other treatments, although Variants 2 and 4 had a higher sugar content, indicating a varied effect of the preparation on the examined grape characteristics.

Keywords: grapevine, stress, biostimulators, sugars, acids

1. Introduction

The grapevine (Vitis vinifera) is an agricultural crop whose primary product, grapes, is consumed as food or processed into various other products. The largest vineyard areas and winemaking centers in Bosnia and Herzegovina are located within the municipalities of the Herzegovina-Neretva Canton. Among the three Herzegovinian wine-growing regions, the Mostar vineyard region is the most significant, accounting for about 90% of the total vineyard area. It is best known for cultivating the indigenous grape variety

Žilavka, which holds great economic importance due to the production of wine that is highly valued both domestically and internationally. Some of the most important agro-biological characteristics of the Žilavka variety include its ripening period in the third epoch, classifying it as a mid-late variety; normal and regular fertilization; and preference for loose, gravelly-stony, moderately fertile, warm, and deep soils. The variety is moderately resistant to low temperatures, more susceptible to downy mildew than to powdery mildew, and during the ripening period, especially in rainy conditions, it is sensitive to gray mold [1].

In addition to soil and climatic conditions, achieving high grape yields and good quality largely depends on temperature and climate factors. The Herzegovina region is particularly suitable for grapevine cultivation due to its high cumulative temperatures during the growing season, exceeding 4,800 °C, and mild winters, where the absolute minimum rarely drops below -10 °C [2]. Temperatures during the ripening period have a significant impact on grape quality, as they influence the key biochemical processes, sugar accumulation, and the breakdown of organic acids. When ripening occurs under high average daily temperatures, grapes generally accumulate a high sugar content, but a larger amount of organic acids is simultaneously degraded. Conversely, under lower temperatures, grapes tend to accumulate less sugar while retaining a higher acid content. Air temperature, combined with adequate moisture, also plays a crucial role in the development of grapevine diseases.

The optimal annual rainfall for grapevine cultivation ranges from 700 to 800 mm, while the minimum required amount is considered to be 400-500 mm of annual water equivalent [2]. The water requirements of grapevines vary depending on the growth stage. Adequate moisture is particularly important during the phase of intense shoot growth and during the period of rapid berry development.

However, excessive moisture can be highly detrimental, as heavy rainfall during flowering and fertilization may result in poor pollination, especially in varieties with functionally female flowers. Associated phenomena, such as drops in temperature and reduced sunlight, can lead to insufficient nutrition of the inflorescences, producing loose clusters. Following a prolonged drought, high rainfall can cause berry skin cracking, which provides ideal conditions for the development of *Botrytis cinerea*. High rainfall is also undesirable during grape ripening and while preparing the vines for the winter period. The occurrence of many grapevine diseases is closely linked to both the amount and the distribution of rainfall during the growing season. For instance, *Plasmopara viticola* can be destructive only in areas with high rainfall or high relative humidity during the vegetation period. The development of *Plasmopara viticola* requires free water on the leaf surface, such as droplets from rain, dew, or fog, or extremely high relative humidity. Infection by *Botrytis cinerea* also directly depends on the presence of water and temperature. Rain negatively affects the development of *Uncinula necator* because it washes away conidia and destroys fungal colonies on infected grapevine organs.

Grapevines require sunlight in all phenophases of development. Light is especially critical for the differentiation of flowering buds, as winter buds developing under good sunlight conditions carry a higher number of potential grape clusters. During ripening, a greater number of sunny days accelerates sugar accumulation, and sun-exposed clusters exhibit better berry coloration. The development of certain pathogens, such as *Uncinula necator* and *Plasmopara viticola*, is influenced by light, although its effect is secondary to temperature and rainfall.

In recent years, due to climatic changes, unfavorable agroecological conditions have been observed during grapevine cultivation, such as high air temperatures and a lack of rainfall.

To mitigate the negative effects, the application of biostimulants is recommended in vineyards that have experienced stressful conditions such as drought, frost, nutrient deficiencies, and plant diseases. Biostimulants used in agriculture encompass a wide range of formulations based on various compounds, substances, and other types of products, including microorganisms, micro- and macronutrients, enzymes, trace elements, plant growth regulators, elicitors, as well as microalgal extracts. These are applied to plants or soils to enhance physiological processes and, consequently, improve crop productivity and quality [3].

Amino acid-based biostimulants or seaweed extracts represent a simple technological measure that can be applied either preventively, before the onset of stressful conditions, or curatively, to enhance plant recovery after damage has occurred. The use of biostimulants such as amino acids and seaweed, targeted foliar fertilization with specific elements (magnesium, copper, potassium), the application of silicon (Si), different types of clay (muscovite and kaolinite), and special polymers that enable water adsorption and retention in the soil has been described [4]. Seaweed extracts, particularly from the brown alga *Ascophyllum nodosum* (L.), are recognized as one of the main groups of biostimulants with significant agricultural potential. Currently, the application of biostimulants of various origins is considered an innovative approach for preventing grapevine diseases while improving grape and wine quality [5]. Biostimulants can be classified as follows: humic substances, seaweed extracts, complex organic materials, amino acids and other nitrogenated compounds, antitranspirants, beneficial chemical elements, inorganic salts including phosphorus, chitin and derivatives of chitosan[18].

According to [6], the use of biostimulants can support sustainable agricultural practices, as their application may enhance nutrient use efficiency, reduce fertilizer losses, increase overall yield, and produce high-quality crops. Their studies demonstrate that biostimulant application positively influences the agronomic performance of olive and grapevine plants by improving olive oil quality and increasing grape yield. Specifically, they reported the beneficial effects of foliar application of seaweed extracts on fruit quality across several black grape varieties under diverse climatic conditions, ranging from cold to warm vineyard regions. This represents the first documented evidence of the positive impact of biostimulants derived from the brown seaweed *Ascophyllum nodosum* on fruit ripening dynamics and quality at harvest [7]. Conversely, other studies have noted that crops treated with biostimulants may respond differently depending on varying climatic conditions [5, 8].

Foliar amino acid-based biostimulants (proline and tryptophan) enhance the photosynthetic activity of plants, helping seedlings quickly overcome slowed growth caused by unfavorable environmental conditions [9]. Table and wine grapes are among the main crops on which biostimulants are applied in Europe [18].

2. Materials and Methods

The research was conducted in 2025 on a family-owned vineyard located in Kočine near Mostar (43.30735, 17.85014). The vineyard, covering an area of approximately 0.1 ha, is planted with the Žilavka variety, situated on gentle slopes and oriented towards the southwest. The planting distance is 1.2 × 1.2 m, and the training system is a double-cordon. The experiment was arranged in a randomized block design with seven variants, each consisting of ten vines. The experimental treatments were as follows: Bioplex, Trazex, Bioplex, AgroK, and Barrier; Bioplex + Inex; Trazex + Agro K; Barrier; Amiksol + Slavol; Aleox; and Control (no treatment).

All treatments were applied using a 20 L SOLO 435 backpack sprayer, and the preparations were used at the recommended concentrations according to the manufacturers' instructions. The treatment schedule for each variant is presented in Table 1.

Table 1. Application schedule of treatments by experimental variants

Variants						
Date	1	2	3	4	5	6
22.04.2025	Bioplex	Bioplex+Inex	Trazex+ Agro K	Barrier	Amiksol+ Slavol	Aleox
10.05.2025	Trazex		Trazex+ Agro K	Barrier	Amiksol+ Slavol	Aleox
25.05.2025	Bioplex		Trazex+ Agro K	Barrier	Amiksol+ Slavol	
27.05.2025		Bioplex+Inex				
12.06.2025	Bioplex		Trazex+ Agro K	Barrier	Amiksol+ Slavol	Aleox
27.06.2025	Bioplex		Trazex+ Agro K	Barrier	Amiksol+ Slavol	Aleox
15.07.2025	Agro K		Trazex+ Agro K	Barrier	Amiksol+ Slavol	
19.07.2025		Bioplex+Inex				
01.08.2025				Barrier	Amiksol+ Slavol	Aleox

Agrotechnical practices were carried out following the standard procedures of the farmer and included, among other measures, soil cultivation with a rotary tiller, the application of copper-based fungicides to control *Plasmopara viticola*, the installation of pheromone traps for the grapevine moth (*Lobesia botrana*), and other treatments. Air temperatures in the vineyard were measured daily using a thermometer, and rainfall was recorded using a rain gauge with a measuring scale. During the growing season, the health status of the grape clusters was assessed on several occasions through visual inspection, based on symptoms and the level of infection expressed as a percentage.

At harvest, yields were determined by weighing the grapes from each vine (10 per variant) as well as recording the total number of clusters per variant. On representative clusters (15 per variant), cluster weight was measured, after which the berries were manually separated from the stems. The berries were collected in separate containers and weighed again, and the weight was recorded. The same procedure was applied to the remaining stems (rachis), which were also weighed separately after separation. In this way, three parameters were obtained for each sampled cluster: total cluster weight, berry weight, and stem weight.

Harvested grape samples for determining total acidity and sugar content were separately packed in plastic bags and transported to the Agromediterranean Faculty laboratory in Mostar, where the analyses were performed. Approximately 1 kg of grapes was sampled from each variant. In the laboratory, the grapes were crushed and gently pressed to obtain must, which was then filtered to separate the clear juice. The obtained juice was used for refractometric measurements, with one drop placed on the prism of the refractometer in three repetitions to ensure reliability and precision of the results. Using the same procedure, samples for determining grape acidity were prepared and analyzed by titration with a sodium hydroxide solution of known concentration in the presence of phenolphthalein as an indicator

All collected data were statistically processed using one-way analysis of variance (ANOVA), which allows for the assessment of whether statistically significant differences exist between groups for all examined parameters. To compare the variants, the LSD test ($p = 0.05$) was applied, enabling the identification of variants that differ statistically and determining the effect of the variants on the mechanical and chemical characteristics of the grapes.

Although Variant 1 (Bioplex, Trazex, Bioplex, Agro K, and Barrier) and Variant 3 (Trazex + Agro K) were included in the experiment, data for these variants could not be collected or included in the statistical analysis due to damage to the clusters caused by flocks of blackbirds.

3. Results and Discussion

For the analysis of climatic conditions during the research period, data from on-site monitoring at the Kočine location were used for the period from March to August 2025. The following parameters were recorded: average, maximum, and minimum air temperature (°C), as well as the amount of precipitation (l/m^2). The data presented in Table

2 show that the highest average and maximum air temperatures were recorded in July, while the minimum temperature was, as expected, the lowest in March. The highest average precipitation was measured in March, May, and August, whereas no rainfall was recorded in June.

Table 2. Average values of temperature and precipitation in 2025

Month	T avg (°C)	T max(°C)	T min(°C)	P avg (l/m2)
March	13.1	17.56	8.03	8.8
April	17.0	21.85	10.64	1.6
May	20.2	25.23	13.41	5.8
June	29.5	35.56	20.12	0
July	30.0	35.7	21.5	1.9
August	27.9	34.3	21.1	4.5

Weather conditions for grape production were favorable, without prolonged drought periods or extreme temperatures. During the summer, high temperatures combined with low air humidity and direct sunlight in Herzegovina can cause damage in the form of sunburn on the berries. Although there was no rainfall in June, the grapevines did not experience drought because there had been significant precipitation in the preceding period, allowing the vines to function normally. At the end of August and the beginning of September, heavy rainfall was recorded on several occasions while the grapes were in full ripening stage.

Air temperature, together with a favorable amount of moisture, has a significant impact on the occurrence of grapevine diseases. The Žilavka variety is sensitive to downy mildew, less to powdery mildew, and extremely susceptible to botrytis, especially during warm and rainy days when infections can be widespread [10]. Regular monitoring revealed the appearance of downy mildew (*Plasmopara viticola*) at the beginning of June, following favorable weather conditions. Considering that only four hours of dew or leaf wetness at suitable temperatures are sufficient for downy mildew infection, the conditions for the development of Plasmopara viticola were favorable at the end of May and the beginning of June, when symptoms were observed on all variants to varying degrees.

The percentage values of downy mildew symptoms on grapevines in June 2025 are presented in Table 3.

Table 3. Leaf damage caused by Plasmopara viticola

Variants						
1.Bioplex, Trazex, Bioplex, Agro K, and Barrier	2.Bioplex+ Inex	3.Trazex+ Agro K	4.Barrier	5.Amiksol+ Slavol	6.Aleox	7.Control
11%	14%	12%	10%	13%	13%	15%

Spring 2025 can be characterized as quite favorable for the development of downy mildew, considering that it rained frequently in May and temperatures remained within the optimal range for downy mildew development. Grapevines from the variants treated with the bioprotector Barrier (1 and 4) had the lowest percentage of leaves showing downy mildew symptoms, while, as expected, the control showed the highest. However, the deviations were very small, so we cannot conclusively determine that the application of biostimulants and bioprotectors had an impact on the occurrence of downy mildew. The grapevine moth (*Lobesia botrana*) was observed, causing some damage to the grapes, but this was insignificant compared to the impact of birds (blackbirds), which, in a short period during August, completely destroyed part of the vineyard, including the two variants closest to their attack. Unfortunately, the grape clusters were completely destroyed or damaged, so we could not carry out measurements on variant 1 (Bioplex, Trazex, Bioplex, Agro K, and Barrier) and the variant with the combination Trazex + Agro K.

3.1 Grape Yield, Mechanical, and Chemical Composition

At the time of technological maturity of the grapes, yields, grape characteristics, as well as sugar and acid content were determined. The results of the measurements of the mechanical composition of the grapes are presented in Table 4.

Table 4. The results of the measurements of the mechanical composition

Variants	Total grape weight per variant (g)	kg/vine	Number of clusters on 10 vines
1. Bioplex, Trazex, Bioplex, Agro K, and Barrier	/	/	/
2.Bioplex + Inex	10.348	1	75
3. Trazex + Agro K	/	/	/
4.Barrier	10.425	1	74
5.Amiksol + Slavol	12.171	1,2	86
6.Aleox	13.329	1,3	96
7.Control	12.601	1,2	89

From Table 4, it can be seen that in the conducted study on 10 vines per variant, differences were observed in the number of grape clusters, yield per vine, and total yield. The lowest number of clusters was recorded in the second (Bioplex + Inex) and fourth (Barrier) variants (75 and 74 clusters, respectively), both of which had a yield slightly above 10 kg per variant. The highest number of clusters was found in variant 6 (Aleox), with a total yield of 13.3 kg, which was also the highest value in the study. The other variants had a total yield of around 12 kg per variant. The average weight per vine ranged from 1 to 1.3 kg.

Grape yields in the Herzegovina-Neretva Canton (HNK) vary from year to year. Table 5 presents statistical data (2020-2024) showing the total yield (t) and yield per vine (kg) of grapes in the Herzegovina-Neretva Canton [11].

Table 5. Grape production in HNK (2020-2024)

Year	Total production (t)	kg/vine
2020	35544	3.5
2021	33533	3.4
2022	40058	3.9
2023		
2024	19271	
27644	1.9	
2.9		

As can be seen from the data presented in Table 5, grape yields in the Herzegovina-Neretva Canton over the observed five-year period were not consistent. The lower yield in 2023 compared to the previous year was caused by unfavorable weather conditions. In the production year 2022, the highest yields were recorded, with a total of 40,058 t and the highest yield per vine of 3.9 kg.

Grape yields in the Municipality of Mostar, according to the same source, over the five-year period 2020-2024 were as follows by year: 8,983 t, 9,405 t, 16,416 t, 4,435 t, while no data for Mostar were available for 2024. The average grape weight per vine in the Mostar area ranged from a minimum of 1.9 kg per vine in 2023 to the maximum yield of 3.9 kg per vine in 2022, which does not correspond with the results from the experimental trial (1 to 1.3 kg per vine).

Based on information from local media as well as conversations with small and large grape producers, it can be noted that vineyard productivity in 2025 increased by 25-30% compared to the previous year.

Numerous factors influence grape yield, including the variety, number of vines per hectare, training system, vineyard age, applied agricultural and viticultural practices, as well as climatic conditions throughout the year.

From the research results and literature data, it can be concluded that grape yields in 2025 were below average, which can be attributed to the age of the vineyard (35 years) and inadequate agronomic practices, which, among other factors, contributed to the occurrence of downy mildew that significantly reduced the yield. Despite the application of biostimulants and favorable weather conditions, the vineyard yield underperformed, indicating to the agricultural producer the inadequate use of protective agents and viticultural practices in the vineyard.

3.2 Cluster, Pedicel, and Berry Weight

To assess grape quality, the proportion of berries and pedicels in the total cluster weight was calculated, as their ratio directly affects yield as well as the technological characteristics of grapes in further processing.

The results of the measurements of cluster, pedicel, and berry weight are presented in Table 6.

Table 6. Summary of basic statistical parameters by variant for cluster characteristics

Variants	Cluster Weight (g)	Pedicel Weight (g)	Berry Weight (g)
2. Bioplex + Inex	148.53a	10.47a	139.33a
4. Barrier	145.93a	9.60b	136.13ab
5. Amiksol + Slavol	140.26b	9.27b	131.00b
6. Aleox	140.66b	9.67b	131.80b
7. Control	147.33a	9.73b	137.67a
$LSD_{0.05}$	6.17	0.76	5.73

The highest cluster weight was recorded in Variant 2 (Bioplex and Inex), which was statistically significantly higher than in Variants 5 and 6, while the difference in cluster weight between Variant 2 and Variants 4 and 7 was not statistically significant. The lowest cluster weight was observed in Variant 5, slightly lower than in Variant 6, and the difference between them was not statistically significant. The highest average pedicel weigh was observed in Variant 2 (10.47 g), statistically highly significant in relation to all the other variants which did not differ from each other significantly.

The highest average berry weight was recorded in Variant 2 (139.33 g), which was statistically significantly higher than in Variants 5 and 6, while it did not differ statistically significantly from Variants 7 and 4.

Table 7. Results of one-way ANOVA analysis for cluster weight

Source of Variations	SS	df	MS	F	P-*vaule*	F *crit*
Between Groups	884.32	4.00	221.08	3.06*	0.02	2.50
Within Groups	5052.26	70.00	72.18			
Total	5936.587	74				

Table 8. Results of one-way ANOVA analysis for stem weight

Source of Variations	SS	df	MS	F	P-*vaule*	F *crit*
Between Groups	11.65	4.00	2.91	2.66*	0.04	2.50
Within Groups	76.53	70.00	1.09			
Total	88.18	74				

Table 9. Results of one-way ANOVA analysis for berry weight

Source of Variations	SS	df	MS	F	P-*value*	F *crit*
Between Groups	798.58	4	199.65	3.21*	0.02	2.50
Within Groups	4350.8	70	0. 62			
Total	5149.38	74				

Observing the tested variants in the experiment, it can be concluded that the best results for all three examined parameters were achieved by Variant 2, that is, the application of Bioplex and Inex.

The results of cluster weight in all variants were below most literature sources, which report cluster weights ranging from 150-200 g [1, 16]. However, some sources indicate that cluster weight can vary within a slightly wider range, from 120-250 g [10]. The application of biostimulants in grape production in Italy increased yield quantity but not grape quality [6]. During a two-year study [12], significant differences in the ampelometric characteristics of the Žilavka variety were observed, with an average cluster weight of 213.05 g in 2014 and 197.80 g in 2015, which is considerably higher than the results of our research. Studies conducted in California, USA, suggest that certain biostimulants improve the quality of table grapes, achieving greater uniformity in berry color and size [17].

3.3 Sugar and acid content

The sugar and acid content in the study of the Žilavka variety in 2025 is presented in Table 10.

Table 10. Chemical properties of Žilavka grape variety in 2025

Variants	Sugar (%)	Acid (g/l)
2. Bioplex + Inex	20.67b	6.2ab
4. Barrier	20.00c	7.4a
5. Amiksol + Slavol	21.77a	5.2b
6. Aleox	20.30bc	5b
7. Control	20.00c	6.2ab
LSD0,05	0.56	1.74

From Table 10, it can be seen that the tested variants of the Žilavka grape variety exhibited different values of sugar and total acid content. The highest sugar content was recorded in Variant 5 (21.77 %), which was statistically significantly higher compared to all other variants. The lowest sugar content (20.00 %) was observed in Variants 4 and 7, with no statistically significant difference from Variant 6. The highest acid content in grapes was found in Variant 4, which was statistically highly significant compared to Variants 5 and 6, while it did not differ statistically significantly from Variants 2 and 7. The lowest acid content was observed in Variant 6, but this difference compared to Variants 2 and 7 was not statistically significant. Tables 11 and 12 present the results of the variance analysis, showing that the tested factor had a statistically highly significant effect on the examined parameters, sugar and acid content (F > F crit).

Table 11. Results of one-way ANOVA for grape sugar content

Source of Variations	SS	df	MS	F	P-*value*	F *crit*
Between Groups	6.48	4	1.62	15.70**	0.00026	3.50
Within Groups	1.03	10	0.10			
Total	7.51	14				

Table 12. Results of one-way ANOVA for grape acidity

Source of Variations	SS	df	MS	F	P-*value*	F *crit*
Between Groups	11.04	4	2.76	2.76**	0.09	3.48
Within Groups	10	10	1			
Total	21.04	14				

At full ripeness, under favorable conditions, grapes accumulate between 20% and 24% sugar, sometimes even up to 28%, while total acids range from 5 to 8 g/l [10]. In a study [13] conducted in 2011 on the Žilavka variety, sugar content ranged from 20.1 to 21.1%, indicating that 2011 was a favorable year for producing high-quality wines. Total acid content in Žilavka grapes ranged from 4.3 to 5.7 g/l, which can be considered low and insufficient for safe processing into high-quality wines. A study of sugar content in Žilavka grapes grown at the Blizanci site in the Mostar wine region showed a significantly higher sugar content in the 2002 harvest (average 20.4%) compared to the 2003 harvest (18.5%). Grapes from the 2003 harvest also had significantly higher total acid content (7.6 g/l) compared to grapes from 2002 (6 g/l) [14]. In another study [15], the total acidity of Žilavka grape must ranged from 6.2 to 6.5 g/l.

4. Conclusion

The application of biostimulants in viticulture is becoming justified due to the negative effects of climate change, as indicated by numerous studies. It is necessary to study their effectiveness on different grape varieties under various climatic and soil conditions.

The weather conditions for grape production in Mostar during 2025 were favorable, without prolonged drought periods or extreme temperatures. However, at the beginning of June, Plasmopara viticola appeared, preceded by favorable weather conditions. Variants 1 and 4, treated with the bioprotector Barrier, showed the lowest percentage of leaf infection, while the highest infection occurred in the control variant 7. The greatest problem was caused by birds in August, which destroyed a part of the vineyard, including two experimental variants that could not be included in the results.

Variant 2 (Bioplex + Inex) performed best in terms of cluster weight, stem weight, and berry weight. Žilavka grapes from the 2025 experiment had sugar content (20-21.7%) and acidity (5-7.4 g/l) consistent with literature data and the reputation of Žilavka as a variety suitable for premium wines. Total yield and yield per vine, as well as the number of clusters per vine, were below average, which can be attributed to the occurrence of downy mildew, the age of the vineyard, and inadequate agrotechnical measures.

This study did not identify a single biostimulant that was superior for all parameters, but it should be noted that it was a relatively small experiment conducted over one year at a single location. Considering also the advanced age of the vineyard and the irregular application of agrotechnical measures, this research can be considered preliminary. Further, larger studies will be conducted to reach more definitive conclusions.

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