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Effect of Biostimulants on Vegetative and Productive Response of Duke Blueberry

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ABSTRACT

This field experiment aimed to assess the effects of algal biostimulants (NPK + TE) and mycorrhiza on the vegetative and productive parameters of a blueberry orchard, as well as the shelf life of fruits at three different stages of harvest. Kosovo benefits from favorable agro-climatic conditions that support high-quality agricultural production. In response to the impact of climate change, the blueberry industry in Kosovo has increasingly adopted Algae biostimulants (NPK + TE) to adapt to changing climate conditions, enhance yields, and improve blueberry resilience against environmental stressors. The experiment was conducted in the Vushtria region on a 6-hectare blueberry orchard using a nested experimental design. The plants were planted at a distance of 1×3 meters, with a density of 3333 plants per hectare in 35-liter pots. The irrigation system used is spaghetti-shaped (4 spaghetti per pot), and the orchard is covered with an anti-hail system. ANOVA analysis revealed significant differences in the number of flowers, number of open flowers, leaf surface, number of fruit, total yield, and canopy volume. The obtained results indicate that the use of mycorrhiza positively affected the number of flowers, number of open flowers, and number of leaves, ultimately increasing yield and canopy volume compared to the use of algal biostimulants (NPK + TE). Additionally, treatment with chitosan at the beginning of the harvest extended the shelf life of fruits to 25 days, significantly longer than fruits treated with algal biostimulants (20 days) and in the control six days.

Keywords: algal biostimulants, mycorrhiza, shelf life, blueberry.

INTRODUCTION

Kosovo generally enjoys highly favorable agro-climatic conditions, conducive to producing high-quality blueberries. The region presents promising marketing prospects in both domestic and export markets, with Kosovo's blueberries reaching the market at least 20 days before the primary competitors in Eastern Europe. The specialty crop industry, particularly in the cultivation of blueberries (*Vaccinium* sp.), shows a keen interest in utilizing biostimulants like humic substances (humic and fulvic acids) and natural seaweed extracts to enhance plant growth and yield. These biostimulants are frequently employed as soil amendments in agriculture and tend to yield optimal results when applied in conjunction with fertilizers. However, the bulk of biostimulant research has focused on annual crops, leaving a gap in understanding their full potential in perennial crops such as blueberries. In earlier studies, it was observed that the use of humic substances led to enhanced plant growth during the initial two years post-planting in blueberries (Vargas, 2015).

The impact of humic substances on root growth was notably significant, resulting in 46–75% greater root dry weights compared to various other treatments, including conventional fertilizers, granular fertilizers, slow-release fertilizers, and a control treatment with the same nutrient composition but lacking humic substances. Additionally, increased growth was observed with the use of humic substances in a new planting of red raspberry (*Rubus idaeus* L). These findings suggest that humic substances are beneficial during the establishment of these crops. However, further research is needed to understand the mechanisms of action and the broader applicability of these products (Bryla & Vargas, 2023).

Chitosan is widely regarded as one of the most promising edible and biologically safe substances, owing to its film-forming properties, antimicrobial actions, lack of toxicity, biodegradability, and biochemical characteristics. Chitosan treatments prolong the shelf life of fruits by reducing the respiration rate, minimizing transpiration loss, and delaying the ripening process (Romanazzi et al., 2017; Kerch, 2015).

In recent years, the blueberry industry in Kosovo has shown a growing interest in using biostimulants to adapt to changing climate conditions, increase yields, and enhance blueberry resilience to environmental stresses. Biostimulants, derived from plant extracts, algal extracts, or beneficial fungi and bacteria, can be applied by both conventional and organic farmers. However, the effects of biostimulants on blueberry production have been inconsistent, underscoring the need for more scientific evidence before widespread adoption. The study aimed to determine the impact of algal biostimulants (NPK + TE) on the vegetative and productive outcomes of Duke blueberry plants, as well as to assess the application of chitosan in the shelf life of the fruit. The application of a method using algal biostimulants (NPK + TE) and mycorrhiza in blueberries represents a contemporary research objective of both theoretical and practical significance.

MATERIAL AND METHODS

The experiment was conducted in the Vushtria region (2023) on a 6-hectare blueberry orchard using a nested experimental design. The plants were planted at a distance of 1×3 meters, with a density of 3333 plants per hectare in 35-liter pots. The irrigation system used is spaghetti-shaped (4 spaghetti per pot), and the orchard is covered with an anti-hail system. The experimental setup involved a nested or hierarchical design, where the categories of the nested factor within each level of the main factor are different. For each treatment with different levels of Mycorrhiza biostimulants; algal biostimulant; Chitosan; Chitosan + Asc. Nodus; Chitosan + Asc. Nodus + algal biostimulant (NPK + TE) + Mycorrhiza and Control). Each treatment used 10 plants, totaling 60 plants per experiment. In the conducted research, Mycorrhiza was applied once to the root system at a dosage of 1 gram per plant. Algal biostimulants (containing NPK + TE) were applied once to the leaves at the onset of flowering and fruit formation, with a dosage of 1.5 ml per plant. Chitosan was administered to the leaves three times, specifically one day prior to harvesting, at a rate of 1.5 grams per plant. Similarly, Chitosan combined with Asc. Nodus was applied to the leaves three times, again one day before harvesting, at a dosage of 1.5 grams per plant (Asc. Nodus was applied once). Each product was applied at the appropriate timing, and for each treatment, the combination of Chitosan, Asc. Nodus, algal biostimulants (NPK + TE), and Mycorrhiza was used in equal amounts as specified above.

The storage capability of the fruits was determined by treating them with the Chitosan-Kitogreen biostimulant (CAS No. 9012-76-4). The plants were treated 24 hours before harvesting with a dose of 0.20 g per plant. For each treatment, 1 kg of blueberries was stored in a refrigerator with a controlled atmosphere at a temperature of 1 °C. At harvest time, at three distinct stages (beginning of harvest on June 27th, 2023, mid-harvest on July 4th, 2023, and end of harvest on July 10th, 2023). To assess the effect of algal biostimulants (NPK + TE), various vegetative and productive parameters were compared, including the number of flowers, number of open flowers, number of leaves, canopy volume, number of fruit, fruit weight, total yield, fruit diameter, and fruit length.

All flowers, open flowers, and leaves were counted. Canopy volume (m^3) was determined by measuring crown dimensions (height, width between rows and in row) at the beginning and end of the vegetation period. In mid-September, the leaf surface area (cm^2) of 20 leaves per plant was measured using the image J software. All fruits on all plants were counted, and their size (diameter of fruit and length of fruit was measured (in mm) at the equator with a caliper (electronic digital caliper) using all fruits per plant at every harvest. The average fruit weight was measured (grams) using an analytical balance for all fruits at each harvest. Yield (in grams per plant) was calculated for all periods of the harvest by measuring the total weight of all fruits per plant.

The data from the measurements were analyzed using a two-way ANOVA with post hoc testing conducted using Stat Plus 2010 from Analyst Soft Inc. USA. Kosovo experiences a medium continental climate with a coastal influence that penetrates through the valley of the White Drin, moderating the typical elements of a continental climate. On the basis of long-term data in Kosovo, the average temperature during the growing (vegetative) season is 16.5 °C, where the hottest month is July (20.1 °C). 744.8 mm of rainfall falls during the year, of which 346.7 mm fall during the growing season, necessitating the need for supplementary irrigation (Zajmi, 1996). The average temperature and the temperature during the growing period were 1–2 °C higher compared to the 30-year average.

RESULTS AND DISCUSSION

The aim of this study was to assess the impact of algal biostimulants (NPK + TE) on blueberry plants and to understand their effectiveness in new plantings. The authors' focus was on evaluating the benefits of several algal biostimulants (NPK + TE), including seaweed extract (Ascophyllum nodosum), mycorrhiza, and chitosan.

The specialized crops industry, including blueberries (*Vaccinium* sp.), is very interested in using biostimulants, such as humic substances (humic and fulvic acids) and natural extracts from seaweed, to improve plant growth and production. These products are typically used as soil additives in agriculture and often perform better when applied with fertilizers. So far, most of the research on biostimulants has been conducted on annual crops (Bulgari et al., 2015; Canellas et al., 2015). Table 1 summarizes the results of the application of algal biostimulants (NPK + TE) on the vegetative parameters of 'Duke' blueberries. Significant changes were observed in all three parameters according to the ANOVA analysis of variance. In terms of the number of flowers, the Mycorrhiza treatment yielded the highest values (255.60), followed by Chitosan, while the lowest values were found in the algal biostimulants (NPK + TE) treatment (160). Regarding the number of open flowers, the control treatment had the highest values (6.01), followed by Mycorrhiza, with the lowest values were found in the Chitosan treatment (5.43). As for the leaf surface, the highest values were found in the Mycorrhiza treatment, while the lowest values were found in the Chitosan + Asc. Nodus + algal biostimulants (NPK + TE) + Mycorrhiza treatment.

Research on the benefits of biostimulants is often mixed and is believed to depend on the conditions under which the products are tested (Rose et al., 2014). Therefore, the obtained results regarding the benefits of using Algae biostimulants (NPK + TE) are specific to the climatic conditions of the orchard where the experiment was conducted. Due to the content of phytohormones such as auxin, gibberellin and cytokinin present in the extract of the algae Ascophyllum nodosum, the diluted application of this preparation on blueberry leaves can be expected to stimulate fruit growth and development, increasing the generation of photosynthates and absorption in soil level of water and nutrients from solution (Gálvez, 2005).

Table 2 presents average data for productive parameters. Significant changes were found in the number of fruits and total yield based on ANOVA analysis. The highest number of fruits was observed in the Chitosan + Asc. Nodus treatment (1504), while the lowest number was in the Chitosan treatment (972). Regarding yield per plant,

Table 1. Average data for vegetative parameters

Treatments	No. of flowers	No. of open flowers	Leaf surface (cm^2)
Mycorrhiza	255.60a	5.95a	16.65a
Algae biostimulants (NPK + TE)	160.00b	5.81a	16.53a
Chitosan	241.00a	5.43b	14.85b
Chitosan + Asc. Nodus	234.00a	5.82a	16.29a
Chitosan + Asc. Nodus + Algae biostimulants (NPK + TE) + Mycorrhiza	187.20b	5.80a	14.37b
Control	202.40c	6.01a	16.11a

Note: The letters in each column indicate significant differences at a level of $P \leq 0.05$ as determined by the LSD test. After 7 weeks of treatment, it was visually evident that the use of Mycorrhiza had a significant impact on plant growth compared to the use of other algal biostimulants (NPK + TE).

Table 2. Average data for reproductive parameters

Treatments	No. of fruits	Fruit weight (gr.)	Total yield/ plant (gr)	Length of fruit (mm)	Diameter of fruit (mm)
Mycorrhiza	1075a	1.806a	2194a	14.39a	15.38a
Algae Biostimulants (NPK + TE)	1022a	1.638a	1605b	14.00a	15.54a
Chitosan	972a	1.878a	1836b	14.05a	15.60a
Chitosan + Asc. Nodus	1504b	1.746a	1842b	14.06a	15.26a
Chitosan + Asc. Nodus + Algae biostimulants (NPK + TE) + Mycorrhiza	958a	1.866a	1787b	14.31a	15.28a
Control	1027a	1.854a	1833b	14.42a	15.05a

Note: The letters in each column indicate significant differences at a level of $P \leq 0.05$ as determined by the LSD test.

the highest values were achieved in the Mycorrhiza treatment (2194 grams per plant), while the algal biostimulants (NPK + TE) treatment had the lowest yield (1605 grams per plant). Table 3 presents the average data for canopy volume at the beginning and end of vegetation, as well as the difference between the first and second measurement. Significant changes were observed in both measurements based on ANOVA analysis.

At the beginning of vegetation, the highest volume was found in the algal biostimulants (NPK + TE) treatment (3.56), while the lowest values were found in the Chitosan treatment (2.60). At the end of the vegetation period, the highest volume was found in the Mycorrhiza treatment (6.21), while the lowest volume was found in the Chitosan treatment (4.49). Regarding the difference between the first and second measurements, the highest values were reached in the Mycorrhiza treatment (3.40), while the lowest difference was observed in the Chitosan treatment (1.89). Table 4 presents the average shelf life data of fruits at three different harvest stages (beginning, middle, and end) for three treatments: Chitosan, algal biostimulants (NPK + TE), and controls. The results indicate that when treated with Chitosan at the beginning of the harvest, the fruits remained viable for 25 days, significantly

Table 3. Average data for canopy volume

Treatments	1st measurement	2nd measurement	Difference between measurements
Mycorrhiza	2.81a	6.21a	3.40
Algae biostimulants (NPK + TE)	3.56b	6.01a	2.45
Chitosan	2.60a	4.49b	1.89
Chitosan + Asc. Nodus	3.22b	5.42a	2.20
Chitosan + Asc. Nodus + Algae biostimulants (NPK + TE) + Mycorrhiza	2.90a	5.24a	2.34
Control	3.00a	6.13a	3.13

Note: The letters in each column indicate significant differences at a level of $P \leq 0.05$ as determined by the LSD test.

Table 4. Average data for shelf life of fruits

Cultivar	Treatments	Date of harverst	Shelf life of fruits/day
Duke	Chitosan	27.06.2023	25
		04.07.2023	17
		10.07.2023	15
Duke	Algae biostimulants	27.06.2023	20
		04.07.2023	14
		10.07.2023	12
Duke	Control	27.06.2023	8
		04.07.2023	6
		10.07.2023	10

longer than those treated with algal biostimulants (NPK + TE), (20 days) and in the control six days. Application of Chitosan on blueberry plants has a positive effect on shelf life, a finding supported by other researchers (Spinardi et al., 2021).

Chitosan treatments have been found to extend fruit life by reducing the respiration rate, minimizing transpiration loss, and delaying the ripening process (Romanazzi et al., 2017; Kerch, 2015). Chitosan has been shown to have a significant effect on reducing the loss of marketable production in various fruits during cold storage. Studies on blueberries (Liu et al., 2018; Mannozzi et al., 2018), strawberries (Feliziani et al., 2015), and table grapes (Meng et al., 2008) have demonstrated its ability to delay and slow down tissue deterioration and decay.

This protective effect is believed to be due to several factors. Firstly, chitosan exhibits direct antimicrobial activity against plant pathogens (Devlieghere et al., 2004; Jiang et al., 2016). Secondly, it has a film-forming activity that acts as a barrier, protecting the fruits from external factors that could lead to decay (Palou et al., 2016). Lastly, chitosan has been shown to stimulate plant defense mechanisms, further enhancing its protective effects.

CONCLUSIONS

On the basis of one year of research conducted on the Duke cultivar of blueberries, focusing on the influence of algal biostimulants (NPK + TE), mycorrhiza, and chitosan on biomorphological parameters in the agroecological conditions of a modern orchard in the village of Maxhunaj, Vushtrri, the following conclusions have been drawn. The Duke cultivar exhibits late flowering, with a flowering duration of 22 days, and early ripening, with a harvest period lasting 23 days. The use of mycorrhiza positively impacted the number of flowers, open flowers, and leaves, ultimately increasing yield and canopy volume compared to the use of algal biostimulants (NPK + TE). Additionally, the application of chitosan extended the shelf life of the efruits after harvest to 25 days, whereas the fruits treated with algal biostimulants (NPK + TE) lasted 20 days, and those in the control group lasted just six days. As an alternative to the excessive use of conventional fertilizers that can pollute the soil and groundwater, the use of algae-based biostimulants could be a very promising option for the future of agriculture in general, and with very effective results as in the conducted experiment, where their use has had a favorable effect on all parameters, both vegetative and productive.

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