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EFFECT OF BIOSTIMULANTS ON DIFFERENT LENTIL VARIETIES IN EASTERN THESSALY (CENTRAL GREECE)

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ABSTRACT

Biostimulants have been useful tools in recent years for increasing crop yields. They are substances or microorganisms that stimulate natural processes in plants, such as root system development, fruit set, and stress resistance. They activate the plant's natural processes rather than providing nutrients directly. In this study, the response of the lentil varieties "Thessaly" and "Samos" in two regions, Magnesia and Larissa (Central Greece), to the application of four biostimulants was examined. Initially, the two fields were prepared and treated, followed by the sowing of the varieties in November. At the beginning of flowering and after reaching 50%, the biostimulants, containing algae extract and amino acids, were applied. The applications were carried out according to the experimental design, which was completely randomized blocks in three replications. Then two samplings followed, one for biomass (ensilage period) and one for seed. The evaluation parameters used were the fresh and dry weight of the plants, the height of the plants, the weight of the seeds, the protein content in the seeds, as well as, in the case of biomass, the content of protein, crude fiber, fat, calcium, phosphorus, ADF, NDF, and ash. The results show that the varieties responded better in quantitative characteristics in the Larissa area, with the "Thessaly" variety recording higher biomass and plant height, while "Samos" exhibited a higher seed yield. Higher values of biomass of 7700 kg/ha resulted from seaweed extract. The highest seed yield was 1617 kg/ha, which resulted from the combination of amino acids and preparation with phosphorus and - potassium.

Keywords: *Biostimulants, lentil varieties, yield, quality characteristics.*

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is a winter legume and is one of the most important crops in the world. Its seeds have a rich nutritional value, as they are a source of protein, minerals, and vitamins essential for human nutrition, while its stems serve as a rich animal feed. The symbiotic relationship develops with nitrogen-fixing bacteria of the genus *Rhizobium*, giving it the ability to bind nitrogen from the

atmosphere, thus improving the nutritional status of the soil (Papakosta-Tassopoulou, 2012).

Lentils are considered one of the oldest crops in the world. Helbeck (1963) states that "the history of lentils goes back to the history of agriculture itself" as the appearance of the plant seems to be closely linked to the beginning of the domestication of wheat and barley by the first farmers of the Neolithic period (Zohary and Hopf 1973). Despite its ancient history, lentils still hold a prominent importance today in various parts of the world, such as the Indian subcontinent, the Middle East, southern Europe, northern and eastern Africa, as well as Australia. It is cultivated to a lesser extent in the New World, except for Canada and the United States, which are among the largest producers worldwide (FAOSTAT, 2022).

Total global production has increased dramatically in the last two decades, disproportionately to the area of cultivation, as yields per hectare have increased significantly. It is indicative that global production has increased by 129% between 2002 and 2022, with the cultivated area increasing by only 50% (FAOSTAT, 2022). The improvement of cultivation practices and the use of new plant nutrition products have contributed to this trend. In the context of using such products that are also environmentally friendly, biostimulants have also been included in recent years.

Biostimulants are defined as substances that can affect plant growth and that are not classified in any existing category of plant protection products or fertilizers. These products consist of one or more substances and microorganisms and can influence certain physiological processes of plant organisms to increase crop efficiency and enhance quality (Calvo et al., 2014). Their mode of action differs from fertilizers and other plant protection products, as fertilizers contribute to the enrichment of the soil with nutrients. Plant protection products aim to protect the plant from pests and diseases, while biostimulants affect the natural processes of the plant to enhance the uptake of nutrients and resistance to abiotic stress (Du Jarbin, 2015). Biostimulants are classified based on the source from which the raw material to be used is obtained. In this way, the main categories are algae and plant extracts, protein and amino acid hydrolysis products, microbial vaccines, humic and fulvic acids, chitosan and other biopolymers, as well as inorganic compounds.

This research aims to investigate the effect of biostimulants on two lentil varieties for the region of Eastern Thessaly (central Greece). To achieve this goal, various biostimulant formulations were applied, and subsequently, a study of the plants' responses to their qualitative and quantitative characteristics was carried out. The varieties evaluated were Thessaly and Samos, which were established in two regions of central Greece.

MATERIALS AND METHODS

Field experiments were established in the growing season from November 2022 to June 2023 in two areas of central Greece : Velestino, Volos (39°23'59.07" N and 22°45'14.62" E), and Psychiko, Larissa (39°26'21.10" N and 22°18'11.60" E). Sowing took place on November 25 and November 15, 2022, respectively. All appropriate cultivation operations were carried out, such as careful soil preparation

before sowing, basic fertilization with the required amounts of phosphorus and potassium for each area according to soil analyses, and application of pre-emergence and post-emergence herbicides, as well as protection from pests and diseases.



Figure 1: Locations of experimental fields in Thessaly plane, central Greece.

A split-plot experimental design was applied with the two varieties as main plots and the six treatments of biostimulants as subplots in three replications ($2 \times 6 \times 3 = 36$ plots) for each region. Each experimental plot was an area of 16 m^2 (4×4), and each experimental field was about 700 m^2 (including margins).

The small-seed variety “Samos” and the large-seed variety “Thessaly” were selected. Biostimulants treatments included: i) M (Control) ii) B1 (*Ascophyllum nodosum* seaweed extract at a dose of 0.07 cc m^{-2}) iii) B2 (glutamic acid, alanine, and aspartic acid at a dose of 0.2 cc m^{-2}) iv) B3 (Combination of preparations B1 and B2) v) B4 (*Ecklonia maxima* seaweed extract at a dose of 0.2 cc m^{-2}) vi) B5 (plant-derived amino acids, fulvic acids, 20% phosphorus and 20% potassium at a dose of 0.2 cc m^{-2}).

The application of biostimulants took place on March 20 for the Velestino area and on March 26 for the Psychiko area.

To evaluate the effect of biostimulants on the growth, development, and yield of the crop, two destructive samplings were carried out, the first on May 9 in Velestino and on May 14 in Psychiko, and the second during the lentil harvest period on June 23 in the Velestino area and June 24 in Psychiko. Measurements were made regarding plant height, fresh and dry weight of the above-ground part of the plants, as well as seed weight.

The NIR analyzer (DA 7250 NIR analyzer, Perten Instruments, Hägersten, Sweden) was used to determine the quality characteristics of the plant samples.

Soil analyses were carried out at a depth of 0-30 cm. The meteorological data came from the meteorological station of the University of Thessaly farm for Velestino and from the meteorological station of the National Meteorological Service in Larissa for Psychiko.

RESULTS AND DISCUSSION

In Velestino, the soil is characterized as clayey with an alkaline reaction in the surface soil horizon. The organic matter percentage is 2.91% with a strong alternative capacity and does not present a salinity problem. In Psychiko, the field is characterized as rich with high concentrations of potassium and magnesium but very low in micronutrients such as iron, zinc, boron, and manganese. The phosphorus content was moderate, and the soil presented a clay-loam texture and an acidic pH. After sowing, weather conditions favored satisfactory germination and initial growth of the crop in both areas (Fig. 2). Then, in January and February, very low rainfall levels were observed, resulting in limited vegetative growth of the plants. Later, the April rainfall was favorable but did not offset the negative consequences of the previous period. The temperature returned to normal levels for the season.

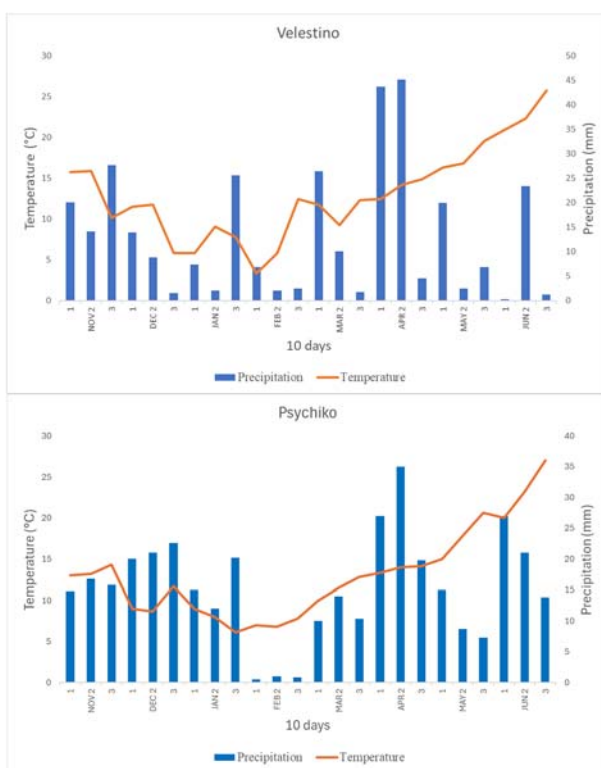


Figure 2: Weather conditions at two regions of experimentation.

Among the lentil varieties, it appears that in both areas, "Thessaly" had better initial growth than "Samos" (Table 1). Seed yields in the two areas are significantly lower than the average yield per acre of the crop for Greece, which ranges from 1200 to 1500 kg/ha depending on the conditions prevailing each year (Theologidou, 2014) This superiority continued until the completion of the plant's biological cycle in Velestino, but the same did not happen in Psychiko, where "Samos" ultimately

gave a higher seed yield, according to the characteristics of the variety as mentioned in the ELGO Dimitra manual (2012). As for the biostimulants, it seems that they favored the cultivation of lentils, giving higher yields both in biomass during the plant's biological cycle and in seed at the end during harvest. In Velestino, the biostimulants only gave a numerical superiority in biomass production, although the difference from the control was about 6000-7000 kg/ha in fresh weight and, on average, over 1200 kg/ha in dry weight in the first sampling. In the same area during the final harvest, biomass production was statistically significantly higher in the biostimulant treatments, from which B4 (*Ecklonia maxima* seaweed extract) stood out, followed by B1 (*Ascophyllum nodosum* seaweed extract) and B3 (combination B1 and B2). The same picture is also presented in the seed yield in the same area. In the mental state, the biostimulants gave a statistically significantly higher yield in biomass, except for B2 (glutamic acid, alanine, and aspartic acid), which was superior only numerically. In the seed yield, the superiority of the biostimulants over the control is again shown. B5 (plant-derived amino acids, fulvic acids, 20% phosphorus and 20% potassium), B4, and B3 stand out as the best interventions. Regarding the interactions between the two factors, the highest yields were given by the variety "Thessaly" with the biostimulants B4 and B3, followed by "Samos" with B1 in Velestino. In Psychiko, the variety "Samos" with the biostimulant B5 gave significantly higher seed yield than almost all other combinations of varieties and biostimulants. It did not differ statistically from the combinations of "Thessaly" with B3 and "Samos" with B4. A similar study by Shehata *et al.* (2017) in the cultivation of beans showed that the application of seaweed extract formulations resulted greater yield in fresh and dry weight compared to amino acid formulations.

Table 1: Plant height, biomass, and seed yield of lentil at two regions of central Greece.

Date		Velestino						Psychiko								
		9/5/2023			23/6/2023			9/5/2023			23/6/2023					
Variables	Plant height	Fresh weight	Dry weight	Dry matter	Dry weight	seed yield	Harvest Index	Plant height	Fresh weight	Dry weight	Dry matter	Dry weight	seed yield	Harvest Index		
															cm	kg/ha
Varieties	Thessaly	37,7	20370	4670	22,9	3310	960	0,29	53,6	23030	5980	26,0	3300	929	0,28	
	Samos	37,8	19410	3720	19,2	2810	830	0,30	41,7	17690	4730	26,7	3436	1117	0,33	
LSD _{.05}		ns	ns	780,0	1,12	276,0	77,0	ns	4,30	2285,0	637,0	ns	ns	86,0	0,031	
Biostimulants	M	37,2	14630	3170	21,7	2140	540	0,25	45,8	14970	4060	27,1	1900	553	0,29	
	B1	38,8	20280	4310	21,3	3400	1100	0,32	43,8	22100	5960	27,0	3267	978	0,30	
	B2	36,5	20550	4300	20,9	2850	840	0,29	49,8	18630	4710	25,3	2780	812	0,29	
	B3	38,8	20570	4280	20,8	3340	940	0,28	50,7	23330	5770	24,7	3633	1178	0,32	
	B4	38,2	21410	4620	21,6	3650	1030	0,28	50,5	21330	5720	26,8	4300	1312	0,31	
	B5	37,0	21910	4400	20,1	2970	930	0,31	45,2	21800	5930	27,2	4320	1305	0,30	
LSD _{.05}		ns	ns	ns	ns	479,0	134,0	0,018	ns	3905,0	1104,0	ns	522,0	149,0	ns	
Varieties X Biostimulants	Thessaly	M	36,7	14240	3390	23,8	2210	560	0,25	51,7	17130	4610	26,9	2067	450	0,22
		B1	39,0	21390	4790	22,4	3030	930	0,31	52,0	28000	7700	27,5	3000	760	0,25
		B2	37,0	19790	4470	22,6	2830	840	0,30	57,0	20000	5000	25,0	2667	657	0,25
		B3	39,3	22080	4940	22,4	4010	1190	0,30	56,3	26270	5950	22,6	4600	1500	0,33
		B4	37,7	24080	5660	23,5	4950	1440	0,29	57,0	22730	5980	26,3	4067	1213	0,30
	Samos	M	37,7	15010	2950	19,7	2070	510	0,25	40,0	12800	3500	27,3	1747	667	0,38
		B1	38,7	19170	3840	20,0	3770	1270	0,34	35,7	16200	4210	26,0	3533	1197	0,34
		B2	36,0	21320	4130	19,4	2880	830	0,29	42,7	17270	4420	25,6	2893	967	0,33
		B3	38,3	19070	3620	19,0	2670	700	0,26	45,0	20400	5580	27,4	2667	857	0,32
		B4	38,7	18730	3580	19,1	2360	620	0,26	44,0	19930	5450	27,3	4533	1410	0,31
B5	37,7	23160	4190	18,1	3130	1060	0,34	42,7	19530	5190	26,6	5240	1617	0,31		
EEA _{.05}		ns	ns	ns	ns	677,0	189,0	0,023	ns	ns	ns	ns	781,0	210,0	ns	
CV %		7,0	25,3	27,0	7,7	13,1	12,5	5,2	13,1	16,2	17,2	10,2	13,7	12,2	15,0	

Concerning the qualitative characteristics, the variety "Samos" shows a slight superiority in biomass during the first sampling (Table 2). In the seed protein, the variety "Samos" statistically excels over Velestino, while the variety "Thessaly" excels in Psychiko. The application of biostimulants does not seem to affect the qualitative characteristics of both biomass and seed protein. There is no distinct difference in the qualitative characteristics from the use of biostimulants, even in the characteristics that show statistically significant differences, such as fat in the biomass of the first harvest in Velestino and seed protein during the final harvest in Psychiko. Furthermore, the interaction of the factors did not show any statistical difference in any of the combinations of the varieties with the biostimulants. Studies by other researchers show an effect of the application of biostimulants on the qualitative characteristics of the biomass and seed of legumes. Ismail *et al.* (2020), report that the application of amino acids to fava bean culture increased protein by 20% compared to the control. In another study by Khairy *et al.* (2011) in fava bean culture, the application of seaweed resulted in a 21.4% increase in crude fiber content. Kocira *et al.* (2020b) in a study on beans, found that the application of seaweed extract recorded the highest ADF content of the application with amino acids provided the best results in NDF.

Table 2. Quality characteristics of biomass and seed protein of lentil at two regions of central Greece.

Date		Velesino									Psychiko									
		9/5/2023									23/6/2023									
Variables	Protein	Ash	ADF	NDF	Crude fiber	Fat	Cal-cium	Phos-phorus	Seed protein	Protein	Ash	ADF	NDF	Crude fiber	Fat	Cal-cium	Phos-phorus	Seed protein		
	Treatments		%									%								
Varieties	Thessaly	13,74	7,35	21,20	39,29	23,24	2,10	0,32	0,23	34,56	13,89	8,28	29,21	50,89	36,20	1,63	0,38	0,21	35,60	
	Samos	14,81	7,76	21,60	40,40	24,34	2,18	0,34	0,25	34,83	14,27	8,43	26,53	47,38	30,05	1,84	0,37	0,24	33,56	
LSD _{.05}		0,670	ns	ns	ns	0,827	0,054	0,054	0,116	0,013	ns	ns	ns	1,480	1,960	2,110	0,063	ns	0,013	
Biostimulants	M	14,07	7,36	20,70	38,47	22,86	2,20	0,32	0,24	36,55	14,17	8,37	27,14	48,03	31,50	1,75	0,36	0,23	33,94	
	B1	14,84	8,03	22,30	41,28	23,92	2,08	0,35	0,25	34,82	13,69	8,12	26,95	47,85	31,60	1,74	0,36	0,22	34,54	
	B2	14,11	7,53	22,60	40,81	24,80	2,08	0,33	0,24	34,52	13,93	8,17	27,65	48,88	33,15	1,76	0,36	0,22	33,96	
	B3	14,64	7,78	21,60	40,36	23,66	2,14	0,34	0,25	34,38	14,85	8,89	29,85	51,74	35,94	1,71	0,39	0,23	35,29	
	B4	13,53	7,17	20,70	39,04	23,74	2,17	0,32	0,23	33,44	13,22	7,93	27,99	49,32	34,18	1,69	0,36	0,22	35,31	
	B5	14,47	7,49	20,70	39,12	23,78	2,20	0,33	0,24	34,48	14,61	8,67	27,63	48,98	32,39	1,74	0,38	0,23	34,45	
LSD _{.05}		ns	ns	ns	ns	ns	0,936	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0,739	
Varieties X Biostimulants	Thessaly	M	13,95	7,49	21,30	38,55	21,88	2,13	0,33	0,24	35,02	14,48	8,71	29,11	50,72	34,61	1,61	0,38	0,23	35,78
		B1	14,17	7,74	22,20	41,03	23,82	2,04	0,34	0,24	35,27	13,02	7,77	27,67	48,79	33,95	1,65	0,36	0,20	35,42
		B2	13,93	7,48	23,00	40,48	24,31	2,06	0,33	0,24	35,54	13,51	8,02	30,08	52,02	37,80	1,63	0,37	0,21	35,39
		B3	13,47	7,21	20,60	38,95	23,16	2,10	0,32	0,23	35,27	15,02	8,77	30,03	51,91	37,33	1,64	0,39	0,22	35,66
		B4	13,15	7,01	20,00	38,03	22,59	2,15	0,31	0,23	33,07	12,77	7,89	30,44	52,51	38,87	1,54	0,37	0,21	35,77
	B5	13,76	7,18	20,40	38,69	23,71	2,15	0,32	0,23	33,21	14,54	8,53	27,91	49,37	34,65	1,70	0,38	0,22	35,56	
	Samos	M	14,19	7,22	20,20	38,38	23,83	2,26	0,32	0,23	38,09	13,86	8,03	25,18	45,34	28,38	1,89	0,35	0,23	32,09
		B1	15,51	8,31	22,40	41,52	24,02	2,13	0,35	0,27	34,37	14,37	8,47	26,23	46,90	29,24	1,83	0,36	0,24	33,67
		B2	14,28	7,58	22,10	41,13	25,29	2,10	0,33	0,24	33,50	14,36	8,31	25,23	45,75	28,50	1,90	0,36	0,24	32,53
		B3	15,80	8,35	22,60	41,77	24,17	2,17	0,36	0,27	33,49	14,69	9,01	29,67	51,58	34,55	1,77	0,39	0,23	34,91
B4		13,90	7,32	21,40	40,05	24,90	2,18	0,32	0,23	33,81	13,68	7,97	25,53	46,13	29,49	1,85	0,35	0,23	34,85	
B5	15,17	7,79	21,00	39,55	23,85	2,24	0,34	0,25	35,75	14,68	8,80	27,35	48,58	30,13	1,78	0,38	0,24	33,33		
EAA _{.05}		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
CV %		6,2	7,0	7,0	4,5	5,0	3,6	5,1	6,8	2,1	8,7	4,8	7,7	5,8	9,2	5,3	6,8	8,9	1,8	

CONCLUSIONS

The lentil varieties did not show their yield potential, possibly due to the weather conditions that prevailed in January and February, with significantly reduced rainfall in the two areas. All applications of biostimulants showed a positive effect on the production of biomass and seeds of the crop. Better results were obtained from combinations of two stimulating preparations such as algae extracts with trace elements and plant proteins with nutrients. The use of biostimulants did not change the qualitative characteristics of the biomass and seeds of the lentil. Repeating the experiment in different environments and time intervals will lead to more reliable conclusions about the effect of biostimulants on the cultivation of lentils.

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