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EFFECTS OF THE BIOSTIMULANTS APPLICATION AT CASTOR BEAN CROP

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Abstract

Castor bean (Ricinus communis L.) is an oil plant of great importance worldwide. It has a very low share in the structure of agricultural crops in Romania, but with a tendency to grow, due to multiple economic advantages.
In the context of climate change new approach to cultivation technology is needed. Improving the effects of stressors is the benefit of using biostimulants. Under this circumstances, the aim of this paper is to present the results of the researchers performed in South Romania with respect to the application of biostimulants at castor bean crop.
In the years 2019, 2020, and 2021, at Agricultural Research and Development Station Teleorman (ARDS Teleorman) located in South Romania, a multifactorial field experiment of type 2 x 2 x 2 was set up, having the following factors: Factor A - biostimulant product: a1 = product containing auxins (Kelpak); a2 = product containing amino acids (Amer Micro); Factor B - application phase: b1 = application at 4-5 leaves; b2 = application before the appearance of the main raceme; Factor C - application rate: c1 = 1 l/ha; c2 = 2 l/ha.
The application of the studied biostimulants determined significant increases in seed yield at castor bean. The obtained results in the three experimental years show that the application of the biostimulants should be done before the appearance of the main raceme and in the rate of 2 l/ha. The effects determined by Amer Micro biostimulant (based on amino acids) was more consistent than those of Kelpak biostimulant (based on auxins).

Key words: *castor bean, biostimulants, auxins, amino acids, seed yield.*

INTRODUCTION

Castor bean (*Ricinus communis* L.) is an oil plant of great importance, which provides oil used as raw material in many industries, as well as used for medicinal and cosmetic purposes, as lubricating oil and biofuel (Ion, 2021).
As raw material, the castor bean oil is used in industries such as those of textiles, leather, machine building and aeronautics, paints, varnishes and emulsions etc. (Sturzu et al., 2014).
Castor bean has a tremendous future potential as an industrial oilseed crop because of its high seed oil content (more than 48%), unique fatty acid composition (90% ricinoleic acid content), and ability to be grown under drought and saline conditions (Severino et al., 2012).
Castor bean plants are able to withstand harsh weather conditions, such as drought, and can be cultivated on marginal lands which are not favourable for cultivation of food crops (Hammad et al., 2019).
In Romania, castor bean is a niche crop, whose share in the structure of agricultural crops is very low, but increasing, due to its advantages: cultivation involves relatively low costs and ensures good yields, with a high oil content; after crop harvest, the land remains with no weeds and the soil is aerated and structured; the plants put very well into value the natural fertility of the soil and the residual effect of fertilizers applied to the preceding crop; the crop capitalizes very well on rainfall and groundwater intake.
In the context of climate change and the emergence of new varieties of castor bean, a new approach to cultivation technology is needed to target the latest concepts and agricultural inputs. A cultivation practice that could be used for improving the performance of castor bean crops is the application of biostimulant products (Santos et al., 2014).
Biostimulants are products capable of acting on the metabolic and enzymatic processes of plants, improving crop productivity and yield quality.

It also helps plants cope with abiotic stress, especially in the early stages of plant development (Xu and Geelen, 2018).

Improving the effects of abiotic stress is the most commonly mentioned benefit of using biostimulants, as 60-70% of production losses in agriculture are estimated to be attributed to abiotic strains (Rouphael et al., 2017; Yakhin et al., 2017).

Biostimulants have positive effects on plant growth, stress reduction and disease prevention, so their use contributes to increasing plant production, yield and quality (Xu and Geelen, 2018).

Studies have shown that foliar application of algae extract leads to a major root development in a variety of species, including corn (Jeannin et al., 1991), tomatoes (Crouch and Van Staden, 1992), strawberries (Alam et al., 2013), and autumn rape (Jannin et al., 2013). Lateral root growths, total root volume, and root length have been observed and attributed to the presence of phytohormones such as auxins and cytokinins in seaweed (Khan et al., 2011a; 2011b).

The chlorophyll content of the leaves increased after the application of seaweed extract in a number of studies. This increase has been associated with a reduction in chlorophyll degradation and delayed senescence, rather than a net increase in the rate of photosynthesis (Jannin et al., 2013).

An innovative technology with promising application potential is the use of hydrolysed proteins (PHs). PHs are "mixtures of polypeptides, oligopeptides, and amino acids that are made from protein sources using partial hydrolysis" (Schaafsma, 2009). They are available as liquid extracts or in soluble powder and in granular form and can be incorporated laterally near the root or applied as foliar sprays (Colla et al., 2015). PHs are produced mainly by chemical hydrolysis (acid and alkaline hydrolysis), thermal and enzymatic hydrolysis of a wide range of both animal waste and plant biomass (Halpern et al., 2015).

Foliar and root applications have been shown to improve the absorption and efficient use of macro- and micronutrients (Halpern et al., 2015). Improved nutrient uptake performance in PHs-treated plants has been largely associated with changes in root architecture (density, length and number of lateral roots), as well as an increase in nutrient availability in the soil solution resulting from nutrient complexation by peptides and amino acids. improved microbial activity (Colla et al., 2015; Du Jardin, 2015).

With the trend towards of using less the chemical fertilizers, biostimulants derived from by-products are expected to be used more frequently if the recovery chain is well established, but the mode of action has to be further investigated.

This paper is aimed to present the results of the researchers performed in South Romania with respect to the application of biostimulants at castor bean crop.

MATERIALS AND METHODS

The researches were carried out at the Agricultural Research and Development Station Teleorman (ARDS Teleorman) located in South Romania (Teleorman county). These were performed under rainfed conditions on a soil of cambic chernozem type, with a loam-clay texture on the depth of the ploughed layer (0-25 cm). From the point of view of physico-chemical properties, the soil is characterized by a content of 45% clay, 3.1% humus, 0.166% total nitrogen content, 40-60 ppm phosphorus mobile, 250 ppm mobile potassium, and by a weakly acid soil reaction (pH = 6.1-6.5).

In the years 2019, 2020 and 2021 a multifactorial field experiment of type 2 x 2 x 2 was set up having the following factors:

- Factor A - biostimulant product, with 2 graduations: a1 = product containing auxins (Kelpak); a2 = product containing amino acids (Amer Micro);
- Factor B - application phase, with 2 graduations: b1 = application at 4-5 leaves; b2 = application before the appearance of the main raceme;
- Factor C - application rate, with 2 graduations: c1 = 1 l/ha; c2 = 2 l/ha.

The field experiments were based on the method of subdivided plots with 3 replications. The surface of the experimental plot was 14 m^2 (L = 5 m, l = 2.8 m).

The technological parameters were the following: the plant density was of 60,000 plants/ha; the distance between rows was 70 cm; the number of plants/plot was 84, and the number of plants/row was 21; the distance between plants/row was 23.8 (cm); the sown variety was Rivlas, which is a mid-late variety created at ARDS Teleorman, Romania; the sowing was performed in the last decade of April.

The biostimulants used in field experiment were Amer Micro and Kelpak.

Kelpak is a pure concentrated biostimulant of seaweed (*Ecklonia maxima*), a very fast-growing algae due to phytohormones (auxins and cytokinins). It contains the optimal ratio of auxins/cytokinins (11 mg equivalent of biological auxins - 0.03 mg equivalent of cytokinins). The application of the Kelpak biostimulant causes the rapid growth of the root tips, which implicitly leads to an increase in the cytokinin content; the development of the root system makes the plants more resistant to certain stressors (drought, waterlogging, nutrient deficiencies, high salt content in the soil, infestation with nematodes and soil pathogens); the better supply of nutrients, correlated with a high level of cytokines in plants, determines the growth and optimal development of crops, and in the end higher yields and better quality are obtained.

Amer Micro has a high content of amino acids (43.8%), which have the role of stimulating root growth and increase tolerance of crops to extreme temperatures, salinity and even drought. Composition: total nitrogen 7.5%, organic carbon 23%, cobalt 0.001%, iron 0.2%, organic nitrogen 7%, zinc 0.01%, free amino acids 16%, boron 0.05%, manganese 0.1%, molybdenum 0.005%, total amino acids 43.8%.

During the vegetation period, phenological observations and biometric determinations specific to castor bean culture were performed.

The seeds were harvested and peeled by hand on each sample.

The calculation and interpretation of the results was made based on the analysis of the variance of the experiments placed in the subdivided plots.

Climatic data. In terms of temperature, during the years of experimentation, castor bean plants benefited during the whole vegetation period of temperatures that satisfied their specific thermal needs (Table 1).

In the pre-sowing months, the average monthly temperatures exceeded the multiannual average of the area in all years of experimentation. April 2021, when the sowing was done, was the coldest in the 3 years of experimentation (9.5°C) compared to the multiannual average of the area (11.9°C). During the period of castor vegetation, the average monthly temperatures exceeded the multiannual average of the area by + 5.3°C on average over the three years of experimentation.

In terms of water, in all the three years of experimentation, in the first part of the vegetation period, respectively in May and June, the precipitations were quantitatively higher than the multiannual average, while in July and August there was registered a significant to severe water deficit (Table 1).

Table 1. Evolution of average monthly temperatures and rainfall at ARDS Teleorman in the experimental years

Average monthly temperature							
Month	II	III	IV	V	VI	VII	VIII
Year 2019	3.5	9.3	11.4	17.0	22.7	23.1	25.2
Year 2020	4.9	8.0	11.9	16.6	21.1	24.7	25.4
Year 2021	3.1	4.7	9.5	17.3	21.0	26.3	25.5
Multiannual average	***-0.5***	***4.6***	***11.9***	***16.8***	**20.6**	**22.6**	**22.4**
Rainfall							
Month	I	III	IV	V	VI	VII	VIII
Year 2019	27.0	26.0	69.8	109.2	171.6	34.8	25.8
Year 2020	68.5	74.0	20.0	79.0	84.0	2.8	0.0
Year 2021	10.5	98.0	36.0	83.0	99.0	1.0	36.0
Multiannual average	***30.9***	***35.0***	***41.8***	***61.2***	**72.6**	**60.9**	**46.8**

RESULTS AND DISCUSSIONS

The application of the biostimulants together with pesticides creates the possibility of the simultaneous execution of several technological links in a single passage of aggregates in the field, thus contributing to the rationalization of energy consumption. In the choice of the application phase of the biostimulants these considerations were taken into account. After the emergence of the main raceme, weeds are no longer a major problem for the castor bean crop, because they no longer have favourable growing conditions (water, nutrients, and especially light), the ground being covered by the castor bean plant canopy. Therefore, after this phenological phase, there is no need to control the weed through technological measures. These ideas were taking into consideration in the establishment of the application phase of the biostimulant products. Also, it was taken into account that the application of the biostimulants could cause an abundant vegetative growth to the detriment of yield, but also a prolongation of the vegetation period, which required a careful observation of the growth and development phenophases.

Plant phenology. Differences in the date of emergence of the main raceme, flowering date, and maturity date were not significant by applying biostimulants compared to the untreated control variant. The number of days from sowing to maturity was on average on the studied variants of 129 days in 2019, 144 days in 2020 and 140 in 2021. Due to climate change, the average monthly temperatures in recent years exceeded in all months of the vegetation period the multiannual average values. As a consequence, the vegetation period of castor bean plants is no longer restricted by the first autumn frosts, this being the reason that early Romanian varieties were created. So, in the present, this aspect is no longer a problem for the castor bean crop in South Romania.

Broken plants is a phenomenon that can cause significant yield losses at castor bean due to the specific architecture of plant. Studies have shown that the use of biostimulants stimulate the development of the root system, which gives the plants a better resistance to breakage.

In order to determine the influence of the biostimulants on the percentage of broken plants, biometric determinations were performed in this respect, in two moments, respectively at the end of the period of formation of the productivity elements and at the physiological maturity of the main raceme. The data are presented as average values of the two determinations (Table 2).

In average over the years of experimentation and for the variants treated with biostimulants, the percentage of broken plants registered the lowest value (3.0%) in 2021, while in 2020 this was higher (3.7%), and in 2019 it was the highest (9.4%), this year being the most humid (Table 2). In 2019, in the first decade of June, when the yielding elements begin to form and the plants are vigorous in their architecture, 114 mm of precipitation fell. The rains were associated with strong storms that broke the castor bean plants, due to the fistulous stem and the fact that the plant's cells were turgid. As the stems were also burdened by racemes, they were prone to breaking. Even after the extreme weather events (storm) of the first decade of June, it was observed that in all experimental variants with application of biostimulants there were fewer broken plants compared to the untreated control.

On average per biostimulant product, the lowest percentage of broken plants had been registered for the variants with Amer Micro biostimulant (based on amino acids). The variants in which the biostimulant product was applied before the appearance of the main raceme had a lower percentage of broken plants compared to the variant of application in the phase of 4-5 leaves. As concerning the application rate, for both biostimulants, the lower percentage of broken plants was registered for the variants with 2 l/ha.

Plant height, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 159.4 cm in the more humid year 2019, while this was of 125.8 cm in 2021 and of 113.0 cm in 2020 (Table 2).

The height of the castor bean plant did not show significant differences in the case of application of the two biostimulant products. However, an increase of the plant height is observed for both products in the case of the application before the appearance of the main raceme and at the application rate of 2 l/ha. On average per biostimulant product, the plant height registered higher values in the case of application of Kelpak biostimulant (based on auxins).

Table 2. Influence of biostimulants on the percentage of broken plants and the plant height at castor bean

No.	Biostimulant	Application phase	Rate (l/ha)	Percentage of broken plants (%) Year 2019	2020	2021	Average	Plant height (cm) Year 2019	2020	2021	Average
1	***Control***	***Untreated***	**-**	***15.7***	***6.2***	***6.5***	***9.5***	***158***	***118***	***126***	***134.0***
2	Kelpak	4- 5 leaves	1	12.5	4.2	3.7	*6.8*	154	108	129	*130.3*
3			2	9.3	3.4	3.1	*5.3*	159	116	127	*134.0*
4		Before appearance of main raceme	1	10.3	5.0	4.2	*6.5*	165	111	122	*132.7*
5			2	6.8	4.2	2.9	*4.6*	168	122	129	*139.7*
Average for Kelpak				***9.7***	***4.2***	***3.5***	***5.8***	***161.5***	***114.3***	***126.8***	***134.2***
6	Amer Micro	4- 5 leaves	1	13.7	5.0	4.1	*7.6*	147	116	121	*128.0*
7			2	10.1	2.5	2.1	*4.9*	155	111	125	*130.3*
8		Before appearance of main raceme	1	6.2	3.4	3.0	*4.2*	164	111	126	*133.7*
9			2	5.9	1.7	1.2	*2.9*	163	109	127	*133.0*
Average for Amer Micro				***9.0***	***3.2***	***2.6***	***4.9***	***157.3***	***111.8***	***124.8***	***131.3***
Average for variants with biostimulants				**9.4**	**3.7**	**3.0**	**5.4**	**159.4**	**113.0**	**125.8**	**132.7**

Main raceme length, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 44.1 cm in the more humid year 2019, while this was of 33.3 cm in 2021 and of 32.1 cm in 2020 (Table 3).

The length of the main raceme registered increased average values for both biostimulants compared to untreated variant.

An increase of the length of the main raceme is observed for both biostimulants in the case of the application before the appearance of the main raceme and at the application rate of 2 l/ha. On average per biostimulant product, the main raceme length registered higher values in the case of application of Amer Micro biostimulant (based on amino acids), with 37.7 cm the length of the main raceme, compared to the application of Kelpak biostimulant (based on auxins), with 35.3 cm the length of the main raceme.

Thousand Grain Weight (TGW), in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 303.6 g in the more humid year 2019, while this was of 229.0 g in 2021 and of 219.7 g in 2020 (Table 3).

The TGW registered increased average values for both biostimulant products compared to untreated variant.

In the case of Amer Micro biostimulant, the higher average value was obtained when it was applied in the rate of 2 l/ha in the phase of 4-5 leaves and in the rate of 1 l/ha before the appearance of the main raceme. In the case of Kelpak biostimulant it was in the contrary, respectively the higher average value was obtained when it was applied in the rate of 1 l/ha in the phase of 4-5 leaves and in the rate of 2 l/ha before the appearance of the main raceme.

Number of capsules on the main raceme, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 78.1 in the more humid year 2019, while this was of 51.0 in 2020 and of 48.4 in 2021 (Table 4).

The number of capsules on the main raceme registered increased average values for both biostimulant products compared to untreated variant.

The highest values of the number of capsules on the main raceme were registered in the case of application of biostimulant products before the appearance of the main raceme and at the application rate of 2 l/ha. Also, the highest values were registered in the case of application of Amer Micro biostimulant (based on amino acids), with 63.1 capsules on the main raceme in average, while the application of the Kelpak biostimulant (based on auxins) determined an average value of 55.3 capsules on the main raceme.

Seed weight on the main raceme, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 81.6 g in the more humid year 2019, while this was of 25.5 g in 2020 and of 24.2 g in 2021 (Table 4).

The seed weight on the main raceme registered increased average values for both biostimulant products compared to untreated variant.

The highest values of the seed weight on the main raceme were registered in the case of application of biostimulant products before the appearance of the main raceme and at the application rate of 2 l/ha. Also, the highest values were registered in the case of application of Amer Micro biostimulant (based on amino acids), with 45.0 g in average, while the application of the Kelpak biostimulant (based on auxins) determined an average value of 42.5 g for the seed weight on the main raceme.

Table 3. Influence of biostimulants on the length of the main raceme and TGW at castor bean

No.	Biostimulant	Application phase	Rate (l/ha)	Main raceme length (cm)				TGW - Thousand Grain weight (g)			
				Year			Average	Year			Average
				2019	2020	2021		2019	2020	2021	
1	***Control***	***Untreated***	-	**47**	**26**	**24**	***32.3***	**305**	**210**	**215**	***243.3***
2	Kelpak	4-5 leaves	1	41	29	27	*32.3*	302	218	228	*249.3*
3			2	45	30	29	*34.7*	303	211	231	*248.3*
4		Before appearance of main raceme	1	42	31	32	*35.0*	300	208	230	*246.0*
5			2	47	35	36	*39.3*	308	228	235	*257.0*
Average for Kelpak				***43.8***	***31.3***	***31.0***	***35.3***	***303.3***	***216.2***	***231.0***	***250.2***
6	Amer Micro	4-5 leaves	1	38	32	34	*34.7*	304	214	220	*246.0*
7			2	43	33	36	*37.3*	306	240	229	*258.3*
8		Before appearance of main raceme	1	49	32	35	*38.7*	306	228	222	*252.0*
9			2	48	35	37	*40.0*	300	210	237	*249.0*
Average for Amer Micro				***44.5***	***32.9***	***35.5***	***37.7***	***304.0***	***223.0***	***227.0***	***251.3***
Average for variants with biostimulants				**44.1**	**32.1**	**33.3**	**36.5**	**303.6**	**219.7**	**229.0**	**250.7**

Table 4. Influence of biostimulants on the number of capsules and the seed weight on the main raceme at castor bean

No.	Biostimulant	Application phase	Rate (l/ha)	No of capsule on main raceme				Seed weight on main raceme (g)			
				Year			Average	Year			Average
				2019	2020	2021		2019	2020	2021	
1	***Control***	***Untreated***	-	**70**	**40**	**38**	***49.3***	**79.0**	**20.0**	**19.7**	***39.6***
2	Kelpak	4-5 leaves	1	70	41	40	*50.3*	79.2	21.4	20.3	*40.3*
3			2	77	43	47	*55.7*	84.2	22.5	21.9	*42.9*
4		Before appearance of main raceme	1	74	48	45	*55.7*	74.4	24.4	23.8	*40.9*
5			2	80	50	48	*59.3*	84.4	28.0	25.3	*45.9*
Average for Kelpak				***75.3***	***45.6***	***45.0***	***55.3***	***80.6***	***24.1***	***22.8***	***42.5***
6	Amer Micro	4-5 leaves	1	75	50	49	*58.0*	82.6	22.8	21.7	*42.4*
7			2	84	60	52	*65.3*	81.9	25.9	24.9	*44.2*
8		Before appearance of main raceme	1	75	50	50	*58.3*	88.1	28.0	27.5	*47.9*
9			2	90	66	56	*70.7*	77.8	30.8	28.3	*45.6*
Average for Amer Micro				***81.0***	***56.4***	***51.8***	***63.1***	***82.6***	***26.9***	***25.6***	***45.0***
Average for variants with biostimulants				**78.1**	**51.0**	**48.4**	**59.2**	**81.6**	**25.5**	**24.2**	**43.8**

Seed yield of any cultivated plant is the result of the interaction of all factors that participate in one way or another in the formation of the harvest. The yield level is in relation to the degree to which each factor and all together come close to the optimal values required by plant biology. This global condition is rare in the natural environment, but it can be improved by combining different agronomic practices: soil preparation, sowing period, plant density, crop rotation, choice of variety or hybrid, fertilization, weed, disease and pest control, equipment used, and the ability to manage vegetation factors so that the “site supply” is as close as possible to the biology of the cultivated plants (Pop et al., 2013).

Seed yield, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 2633.5 kg/ha in the more humid year 2019, while this was of 1422.5 kg/ha in 2020 and of 1307.9 kg/ha in 2021 (Table 5). Compared to the seed yield obtained in 2019, on average for the study years, there is a difference of -1211 kg/ha in 2020 and of -1325.6 kg/ha in 2021. This difference is due to the climatic conditions of 2020 and 2021, when the drought was installed during the formation of the yielding elements.

The seed yield registered increased values for both biostimulant products compared to untreated variant in all experimental years and in all application phases and rates.

The highest values of the seed yield were registered in the case of application of biostimulant products before the appearance of the main raceme and at the application rate of 2 l/ha. Also, the highest values of the seed yield were registered in the case of application of Amer Micro biostimulant (based on amino acids), with 1855.3 kg/ha in average, while the application of the Kelpak biostimulant (based on auxins) determined an average value of 1720.6 kg/ha. Amer Micro biostimulant determined an average seed yield increase of 134.7 kg/ha compared to Kelpak biostimulant.

Table 5. Seed yields obtained by application of biostimulants on castor bean

No	Biostimulant	Application phase	Rate (l/ha)	Seed yield (kg/ha)			
				Year			Average
				2019	2020	2021	
1	***Control***	***Untreated***	-	**2289**	**1187**	**935**	***1470.3***
2	Kelpak	4-5 leaves	1	2341	1281	1120	*1580.7*
3			2	2511	1319	1210	*1680.0*
4		Before appearance of main raceme	1	2594	1339	1278	*1737.0*
5			2	2833	1441	1380	*1884.7*
Average for Kelpak				***2569.8***	***1345.0***	***1247.0***	**1720.6**
6	Amer Micro	4-5 leaves	1	2409	1367	1271	*1682.3*
7			2	2838	1461	1387	*1895.3*
8		Before appearance of main raceme	1	2465	1501	1345	*1770.3*
9			2	3077	1670	1472	*2073.0*
Average for Amer Micro				***2697.3***	***1499.9***	***1368.8***	**1855.3**
Average for variants with biostimulants				**2633.5**	**1422.5**	**1307.9**	**1787.9**

In order to determine the influence of each factor, but also their interaction on the seed yield of castor bean, the analysis of variance for the multifactorial experiment with 2 biostimulant products x 2 application phases x 2 application rates was performed for each year of study (Table 6). Analysing the variance table for the seed yield of the multifactorial experience of type 2 x 2 x 2, one observes the very significant influence of factors A (biostimulant product), B (application phase) and C (application rates) in the years of experimentation 2019 and 2020, and the distinctly significant influence of these factors in 2021. In 2019, the interactions between experimental factors were very significant, and the triple interaction A x B x C significant, but in 2020 and 2021, due to less favourable climatic conditions for castor bean crop interactions between factors were insignificant.

Table 6. Analysis of variance (ANOVA) for seed yield on the multifactorial experiment with biostimulants (2 biostimulant products × 2 application phases x 2 application rates)

Source of Variation	Sum of Squares (SS)			DF*	Mean Squares (MS)			F-test		
	2019	2020	2021		2019	2020	2021	2019	2020	2021
Biostimulant	97282.7	169680.2	89182.0	1	97282.7	169680.2	89182.0	106.6***	77.7***	30.6**
Application phase	282968.2	466488.2	89182.0	1	282968.2	466488.2	89182.0	545.0***	204.9***	11.2**
Biostimulant x Application phase	29540.2	3408.2	10710.4	1	29540.2	3408.2	10710.4	56.9***	1.5^{NS}	1.3 ^{NS}
Application rate	462592.7	153920.2	71177.0	1	462592.7	153920.2	71177.0	218.6***	44.3***	15.9**
Biostimulant x Application rate	353322.7	1504.2	975.4	1	353322.7	1504.2	975.4	167.0***	0.4^{NS}	0.2 ^{NS}
Application phase x Application rate	131128.2	6080.2	198.4	1	131128.2	6080.2	198.4	62.0***	1.8^{NS}	0.04 ^{NS}
Biostimulant x Application phase x Application rate	19153.5	11008.2	0.04	1	19153.5	11008.2	0.0	9.1*	3.2^{NS}	0 ^{NS}

*DF - Degrees of Freedom

Comparing the average seed yields per biostimulant product, one notices that in 2019 the Amer Micro biostimulant determined a distinctly significant increase in seed yield of 127.5 kg/ha, compared to the seed yield obtained in the case of Kelpak biostimulant (Table 7). Significant increase of the seed yield of 154.9 kg/ha and of 121.8 kg/ha was also obtained in 2020 and respectively in 2021 when applying the Amer Micro biostimulant, compared to the seed yield obtained in the case of Kelpak biostimulant.

Comparing the average seed yields per application phase of the two biostimulants, one notices that in 2019 and 2020 the application before the appearance of the main raceme determined very significant increase in seed yield of 217.5 kg/ha and respectively of 130.75 kg/ha, compared to the seed yield obtained in the case of application in the phase of 4-5 leaves. But in 2021, despite the fact that the seed yield registered an increase by applying the biostimulant products before the appearance of the main raceme compared to the application in the phase of 4-5 leaves, the yield increase of 121.75 kg/ha was not significant.

Comparing the average seed yields per application rate of the two biostimulant products, one notices that in 2019 application of 2 l/ha determined a very significant increase in seed yield of 362.5 kg/ha, compared to the seed yield obtained in the case of application of 1 l/ha. Distinct significant increase of the seed yield of 100.8 kg/ha and of 108.8 kg/ha was also obtained in 2020 and respectively in 2021 in the case of application of 2 l/ha compared to the application of 1 l/ha.

Table 7. Limit differences for the influence of experimental factors on castor bean seed yield

LSD (kg/ha)	For biostimulant			For application phase			For application rate		
	2019	2020	2021	2019	2020	2021	2019	2020	2021
5%	52.9	83.5	94.7	25.9	54.2	202.3	43.4	55.6	63.0
1%	122.2	170.8	218.7	42.8	89.6	334.8	63.2	80.9	91.6
0.1%	389.0	470.7	695.8	80.2	167.7	626.6	94.8	121.3	137.4

In 2019, an increase of 287.5 kg/ha was obtained when the Kelpak biostimulant was applied before the appearance of the main raceme, and an increase of 147.5 kg/ha when the Amer Micro biostimulant was applied before the appearance of the main raceme, compared to application in the phase of 4-5 leaves. Also compared to application in the phase of 4-5 leaves, in 2020, the Kelpak biostimulant applied before the appearance of the main raceme determined an increase of 90 kg/ha, and the Amer Micro biostimulant an increase of 171.5 kg/ha, while in 2021 the application of the Kelpak biostimulant applied before the appearance of the main raceme determined an increase of 164 kg/ha, and the

Amer Micro biostimulant an increase of 79.5 kg/ha.

Analysing the seed yields obtained when applying the same biostimulant product but in different application rates, it is observed that in 2019, the increase of the Amer Micro biostimulant application rate from 1 l/ha to 2 l/ha brings a very significant yield increase of 520.5 kg/ha, while in the year 2020 and 2021 the increase was distinctly significant, with 131.5 kg/ha and respectively 121.5 kg/ha. Increasing the application rate of Kelpak biostimulant from 1 l/ha to 2 l/ha brings a significant increase in seed yield only in 2019, with 204.5 kg/ha.

Analysing the seed yields obtained at the same application phase of Kelpak biostimulant, but at different application rates, it was found that at the application phase of 4-5 leaves, increasing the application rate from 1 l/ha to 2 l/ha brings a significant increase in seed yield only in 2019 (170 kg/ha). At the application stage before the appearance of the main raceme, increasing the application rate from 1 l/ha to 2 l/ha brings a very significant increase in seed yield in 2019 (239 kg/ha) and significantly increase in 2020 and 2021 (102 kg/ha in both years of experimentation).

Analysing the seed yields obtained at the same application phase of the Amer Micro biostimulant, but at different application rates, it was found that at the application phase of 4-5 leaves, increasing the application rate from 1 l/ha to 2 l/ha brings a significant increase in seed yield in 2019 (429 kg/ha) and 2021 (116 kg/ha). At the application stage before the appearance of the main raceme, increasing the application rate from 1 l/ha to 2 l/ha brings a significant increase in seed yield in all years of experimentation (612 kg/ha in 2019, 169 kg/ha in 2020, and 127 kg/ha in 2021).

Table 8. Limit differences for all combinations of experimental factors on castor bean seed yield

LSD (kg/ha)	Interactions											
	a1b2-a1b1			a2b1-a1b1			a1c2-a1c1			b1c2-b1c1		
	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
5%	36.6	76.6	143.1	57.9	83.5	126.2	61.4	78.6	89.1	61.4	78.6	89.1
1%	60.6	126.7	236.7	153.3	170.8	238.6	89.3	114.4	129.5	89.3	114.4	129.5
0.1%	113.4	237.1	443.1	359.2	470.7	584.7	134.0	171.6	194.3	134.0	171.6	194.3
	Interactions											
LSD (kg/ha)	b2c2-b1c2			a2c2-a1c2			a1b1c2-a1b1c1			a1b2c1-a1b1c1		
	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
5%	59.1	92.9	105.7	108.2	132.9	151.2	32.6	32.6	72.5	83.6	83.6	125.1
1%	65.8	183.7	209.0	120.0	147.3	167.7	47.5	47.5	105.5	93.0	93.0	139.2
0.1%	103.6	482.3	548.9	293.1	359.9	409.6	71.2	71.2	158.2	146.6	146.6	219.4

CONCLUSIONS

The application of the studied biostimulants determined significant increases in seed yield at castor bean.

The studied biostimulants did not extend the growing period of the castor bean plants.

The application of the studied biostimulants before the appearance of the main raceme and in the rate of 2 l/ha reduces the percentage of broken plants. The Amer Micro biostimulant (based on amino acids) determined the lowest percentage of broken plants and a less effect on the plant height.

Also, the application of the studied biostimulants before the appearance of the main raceme and in the rate of 2 l/ha determined an increase of the length of the main raceme, of the number of capsules on the main raceme, of the seed weight on the main raceme, and finally of the seed yield.

The obtained results in the three experimental years show that the application of the studied biostimulants should be done before the appearance of the main raceme and in the rate of 2 l/ha.

The effects determined by Amer Micro biostimulant (based on amino acids) was more consistent than those of Kelpak biostimulant (based on auxins).

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